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Studies on the pathogenicity, morphology, and cytology of Nematospora Phaseoli *

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(WITH PLATES 7–9)

INTRODUCTION

As a group, the yeasts have been regarded primarily as typical saprophytes which set up fermentation changes by the action of their enzymes upon carbohydrate materials. However, the classic paper of Metchnikoff ('84) on phagocytosis describes a yeast parasitic on the crustacean *Daphnia*, and more recently certain yeasts have been found to cause disease in plants.

Our knowledge of the cytology of the yeasts is based on the studies of comparatively few investigators, whose results have been to a large extent contradictory.

My studies have been directed (1) to the morphology and phylogenetic relationships of the group of yeasts with slender elongated ascospores; and (2) to the cytology of the ascus in *Nematospora Phaseoli*.

MORPHOLOGY AND PHYLOGENETIC RELATIONSHIPS OF THE GENUS NEMATOSPORA

The genus *Nematospora* is one of a series of yeast genera which are characterized by long needle-shaped ascospores. This series of genera includes *Nematospora*, *Monospora*, and *Coccidiascus*. Whether such a series represents a phylogenetic unit is difficult to determine; but this is strongly suggested by the

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morphological characteristics of these genera. Moreover, the similarity of their habitats is very suggestive.

The genus *Nematospora* was created by Peglion in 1897, when he described the species *N. Coryli*. This species, according to Peglion, multiplies by budding in the cotyledons of hazel nuts and upon solid culture media, and produces asci from individual bud cells. In liquid media budding ceases and a sterile mycelium is produced. The asci are cylindrical, 65–70 microns long by 6–8 microns wide, and contain eight needle-shaped ascospores arranged in groups of four in each half of the ascus. The ascospores are one-celled, 2–3 microns wide by 38–40 microns long, and are equipped with a long flagellum-like appendage at one end. This appendage disappears during germination and the ascospore assumes the form of a short cell that may produce buds at both extremities.

The species *N. Lycopersici* described by Schneider ('16) from tomato fruits, although agreeing with *N. Coryli* in its most essential characteristics, differs from it in certain respects. In *N. Lycopersici* the asci, according to Schneider, arise from a conjugation of two bud cells, and there is evidence that they may develop from single cells, especially in vigorously growing cultures. The ascospores agree in general in their structure and method of germination with those of *N. Coryli*.

Nowell ('17) described what he considered as four further species of *Nematospora*. He designated these as *A*, *B*, *C*, and *D*. *Species A* and *B* in my opinion should not be regarded as species of *Nematospora*. They never multiply by budding, according to Nowell, and their so-called sporangia contain a very large number of spores which show a wide divergence in size and other characteristics from the ascospores of *Nematospora*. As Nowell points out, *Species B* agrees closely with the description of *Eremothecium Cymbalariae* as given by Borzi and Arnaud, and I believe he is justified in considering it the same or a very closely related species. The agreement is most marked in the matter of the number and arrangement of the spores in the so-called sporangium. Nowell's *Species C* and *D* bear a very close resemblance to the *Nematospora* species of Peglion and Schneider, and in my opinion clearly fall under the genus *Nematospora*. The ascospores are formed in two equal bundles in the opposite halves of the ascus. They possess the general characteristics of

the ascospores of *Nematospora Coryli* and *N. Lycopersici*, including the whip-like appendage. In germination the upper half of the ascospore (remote from the appendage) takes no visible part in the process. Nowell denies the existence of a septum at the middle region of the ascospore and yet he states that at the time of germination the ascospore wall just below its middle region swells into a sphere, from which germination takes place. This germination may be in the form of a germ tube or in the form of yeast-like budding, depending upon the conditions under which the ascospores are germinated.

The asci in *Species C* are intercalary in the cells of the hyphae. In *Species D* the asci may be formed by growth of the cells of the hyphae, but they are usually produced from single bud cells. The ascospores in this species sometimes germinate directly into asci. The thallus of *Species D* is typically yeast-like, whereas that of *Species C* is a dichotomously branched mycelium.

The genera *Monospora* and *Coccidiascus* have certain characteristics in common with the genus *Nematospora*. *Monospora bicuspidata*, *the only species of this genus, was described by Metchnikoff ('84) from the body cavity of a *Daphnia* species, where it multiplies as a yeast. Its long cylindrical asci arise from the individual yeast cells and contain a single needle-shaped ascospore. The germination is like that in *Nematospora*, that is, a chain of yeast cells is produced from the middle region of the ascospore. The ascospores are apparently one-celled.

Coccidiascus Legeri multiplies by ordinary yeast budding and lives as a parasite in the intestinal cells of *Drosophila funebris*. The banana-shaped asci apparently arise from the conjugation of two yeast cells, and contain eight needle-shaped ascospores

*This specific name was written, apparently through inadvertence, as *Monospora cuspidata* Metchn. by Zopf (Die Pilze, 1890) and the error has been perpetuated by Lindner, Saccardo, and others down to Guilliermond and Tanner ('20). It is explicitly stated by Metchnikoff ('84, p. 181) that he adopted the specific name *bicuspidata* because of the fact that the spores are pointed at both ends, and this original form of the name is confirmed by Madame Olga Metchnikoff in her biography of her husband, both in the French edition and in her English translation (London, 1921). As this is the sole species of the genus, there could be no valid excuse for changing the specific name. Metchnikoff's generic name is antedated by the algal genus *Monospora* Solier (1845) of the Ceramiaceae, but even in that genus no specific name resembling *bicuspidata* is recorded. EDITOR.

arranged side by side in a single group. The ascospores of this species, like those of *Monospora bicuspidata*, are one-celled and without an appendage.

Other organisms that resemble *Nematospora* in one or more points are *Eremothecium Cymbalariae* Borzi ('88) with slender spores arranged in two opposite bundles in the so-called sporangium; *Protascus subuliformis* Dangeard ('03) with long slender spores, and a somewhat similar arrangement in the so-called sporangium; *Endomyces capsularis* Schiønning ('03) with asci formed intercalary in the mycelium, and ascospores that germinate either by budding or by sending out a germ tube, or, under special conditions, by producing an ascus direct; and *Endomyces javanensis* Klöcker ('09) with asci that seem to form indifferently from yeast cells or at the expense of some cell in the mycelium, and ascospores that germinate either by budding or by forming a germ tube.

Five species of *Nematospora* have been described, namely, *N. Coryli* Peglion, *N. Lycopersici* Schneider, *N. Species C* and *D* Nowell, and *N. Phaseoli* Wingard. These species show a remarkably close similarity in all their morphological characteristics, and the few minor differences that exist may fall well within the limits of the variations of species. In the genus *Monospora*, with the species *M. bicuspidata* Metchnikoff, and in the genus *Coccidiascus*, with the species *C. Legeri* Chatton, one finds forms that are very similar, both in their yeast-like development and in the character of ascospores produced. I am, therefore, convinced that these genera as noted are closely related to the genus *Nematospora*.

The genera *Protascus* and *Eremothecium* bear some resemblance to the genus *Nematospora*, but they must be studied more critically before one can be justified in making a definite statement as to their taxonomic position.

We are now confronted with the question as to whether the genus *Nematospora* should be referred to the *Saccharomycetes*. Peglion ('97, '01) in establishing the genus, did not hesitate to assign it to the *Saccharomycetes*. Hansen ('04) however, placed it with the genus *Monospora*, and considered both genera as doubtful *Saccharomycetes*. Schneider agrees with Peglion as to the relationships of the genus; Nowell, on the other hand, regards the fruiting body of *Nematospora* as a sporangium, and for that

reason states that the genus should not be referred to the *Saccharomycetes*. Guilliermond ('12) places the genera *Nematospora*, *Monospora*, and *Coccidiascus* in the fifth group of his classification of the *Saccharomycetes*, and designates them as budding yeasts with uncertain affinities.

My cytological studies of *Nematospora Phaseoli* show that its spore bearing body is undoubtedly an ascus. It seems, therefore, that Nowell's argument against placing the genus under the *Saccharomycetes* may be disregarded. Furthermore, practically all of the characteristics of *Nematospora* find parallels in one or more genera of the *Saccharomycetes*. I thoroughly agree, then, with Guilliermond in referring the genera *Nematospora*, *Monospora* and *Coccidiascus* to the *Saccharomycetes*.

Morphological studies on Nematospora Phaseoli

My own observations confirm the general conclusions just indicated as to the characters and relationships of this group of yeast-like genera.

As previously described (Wingard, '22) *Nematospora Phaseoli* was isolated from diseased Lima beans (*Phaseolus lunatus*) from eastern Virginia in 1921. A number of isolations have been made from Lima beans from different parts of Virginia and some of these isolations have been maintained in pure culture for more than three years. Monospore cultures were obtained by the use of the Barber pipette, and all reasonable precautions have been taken to keep the cultures pure.

Methods

Beer wort agar* has been most generally used in my culture studies of *Nematospora Phaseoli*. For poured plates and slants 10 to 15 cc. of this medium were employed. In the study of giant colonies 50 cc. were placed in 150 cc. Erlenmeyer flasks. The vegetable slants were cut out with a three-eighths inch cork

* Beer wort agar is prepared as follows: Soak 200 grams of crushed malt in a liter of cold water and slowly heat to a temperature of 60° C. Shake once in a while and after 45 minutes add 4 grams of hops; boil for an hour and filter. Add distilled water to make the filtrate up to a liter and then add 15 grams of agar agar, and gradually bring it into solution. Filter, and sterilize in autoclave at 15 pounds pressure for 20 minutes. The beer wort is the same as the above minus agar agar.

borer. All vegetable material was sterilized in the autoclave at 15 pounds for 20 to 30 minutes, with the exception of tomato fruits which were used uncooked.

Beer wort agar and fresh slants of garden beet, sweet potato, Irish potato, turnip, radish, carrot, parsnip, and green snap bean pods are very favorable media for both vegetative growth and ascospore formation. Sliced ripe tomato gives a fairly good growth. Whey agar, beef peptone agar, Czapek agar, corn meal agar, and Pfeffer's nutrient solution, with or without sugar, plus 1.5 per cent agar are very poor media. Of the liquids tested, beer wort, tap water and distilled water were found to give the best development. Pfeffer's nutrient solution gave no growth at all.

The vegetative phase of this species is typically yeast-like as a rule, but under certain conditions a mycelium is produced.

On beer wort agar plates at a temperature of 28 to 30° C., the colonies appear in 36 to 48 hours. They are convex, circular, with entire margins and smooth surface, opaque, dull. The internal structure is finely granular. The colony is cream colored at first but gradually turns brown with age. The maximum diameter of the colony is about 18 mm. and is attained in four to seven days. Asci and ascospores are produced in great numbers in 48 to 72 hours. After about a week the colony becomes surrounded by a border of mycelial growth, resulting from the germination of ascospores within the colony.

On beer wort agar slants, growth is abundant, slightly filiform in appearance, raised, dull, cream colored, opaque, contoured, with a butyrous consistency. The entire slant is covered in about a week. Mycelial branches develop on the sides of the test tube surrounding the margin of the slant. Giant colonies, grown on 50 cc. lots of beer wort agar in 150 cc. Erlenmeyer flasks, attain a diameter of 75 mm. in 30 days.

Growth in liquids is not so abundant as it is on solid media. In beer wort (solution) the growth is fairly slow but may apparently take place either on the surface or at the bottom of the liquid. In some cases a ring is formed on the sides of the test tube just at the surface of the liquid and the least jar is sufficient to cause the cells to settle to the bottom of the tube. In other cases the growth apparently takes place at the bottom of the tube, no development being visible at the surface of the solution.

The growth in beer wort is typically yeast-like, but occasionally short mycelial filaments can be found. Practically no asci and ascospores are developed.

In distilled water and also in tap water, a flocculent growth is made at the bottom of the liquid. This is composed almost entirely of mycelial filaments. Some are sterile and only rarely septate, while others are septate and bear intercalary asci which contain ascospores. The growth in very dilute beer wort is very similar to that in distilled water, but more yeast cells are produced than in the latter.

The organism produces a distinct alcoholic odor when grown in beer wort, and on beer wort agar and also on the vegetable media mentioned above.

The yeast cells multiply by budding, and it is not uncommon to see fifteen to twenty cells still attached to each other. The cells vary from a small ellipsoid type to large oval ones. The ellipsoidal cells measure from 5 to 14 microns by 2 to 10 microns and predominate in young cultures. The spherical cells measure from 10 to 20 microns in diameter and are very numerous in old cultures. They develop from the small ellipsoidal cells, and correspond to the arthrospores of Schneider.

Asci and ascospores are produced in great numbers in the cotyledons of Lima beans and also in favorable culture media such as beer wort agar or slants made from sweet potato, garden beet, etc. The asci arise from individual bud cells. They are cylindrical with rounded ends, 60–85 microns by 10–12 microns; ascospores eight, in two groups of four, 40–46 microns by 2.5–3 microns, slender, 1-septate, slightly ridged at septum, apex acute, base extended into a slender non-motile appendage about one and one-fourth times the length of the ascospore. In old cultures and sometimes under other conditions less than eight ascospores are formed in the asci.

Temperature relations

The minimum temperature for the growth of *N. Phaseoli* on beer wort agar is about 15° C.; the optimum, 25 to 30° C.; and the maximum, 40° C. The minimum temperature for ascospore formation is about 18° C.; the optimum, 25 to 30° C.; and the maximum, 34° C. Cultures grown at a temperature of 35 to 37.5° C. produce a fine type of yeast from the standpoint of its

budding. These cultures lose their spore-forming power if subjected to this temperature for a very long time. The same thing occurs when a culture is left too long before being transferred to fresh media. Two of my cultures were allowed to go for about a month on beer wort agar slants before being transferred. They lost the power to form spores in that short time. These cultures have been grown on various media for the last two years but not a single spore has been observed. They produce mycelium under the conditions described above, but they multiply primarily by budding as true yeasts.

PATHOGENIC YEASTS

The discovery of parasitism among the yeasts should perhaps be credited to the French physician, Dr. Maurice Raynaud ('69), who described and figured a yeast like organism, which he compares with *Oidium albicans* and *Saccharomyces Cerevisiae*, as regularly found in connection with the malady called black tongue. Raynaud raised the question as to whether the yeast actually caused the hypertrophy, but expressed the opinion that the latter merely furnished a condition favorable for the growth of the parasite. It is probably the same organism which was described as *Saccharomyces linguae-pilosae* by Lucet ('01) in an account of this disease. According to Guéguen ('09) and Thaon ('09), this yeast acts only in association with *Oospora lingualis*. There is said to be a symbiotic association between these two organisms.

Metchnikoff ('84) in the paper referred to above, reported the discovery of the yeast *Monospora bicuspidata* in the body cavity of a crustacean (*Daphnia*) causing a typically diseased condition. The transparency of this crustacean enabled Metchnikoff to follow with great accuracy the interrelation of host and parasite, and the entire process of infection. He thus made the memorable discovery of phagocytosis which has since played such an important rôle in medical science.

Since the appearance of Metchnikoff's report, numerous other yeasts and yeast-like fungi have been reported as parasites on both man and animals. *Endomyces albicans*, according to Guilliermond ('12), has been found to produce lesions in man. It is now supposed to be the causal agent of the disease known as thrush, and this or a similar organism has also been found in

lung infections by Simon ('17), Birch-Hirchfeld ('75) and Castellani ('13). Ashford ('15) in studying the etiology of the disease known as sprue has announced the constant presence of a yeast like organism in the digestive tract. He thus confirms and extends the results of Bahr ('14), Kohlbrugge ('01), Le Dantec ('08) and others, all of whom had previously isolated fungi of the *Oidium albicans* type from sprue patients. Ashford's organism was named *Parasaccharomyces Ashfordi* by Anderson ('17) who also isolated another species, similar in physiologic properties, but differing in morphologic characteristics. The yeast-like organism, *Cryptococcus farciminosus*, discovered by Rivolta ('73) and more thoroughly studied by Rivolta and Micellone ('83) as the cause of farcy or African glanders in horses and mules, has been discussed by several other investigators in southern Europe and Japan.

A number of yeasts have been described in tumors of various kinds. One of the most characteristic of these is the yeast *Saccharomyces subcutaneus tumefaciens*, described by Curtis ('96) from a tumor of the hip and also from a lumbar abscess in man. *Saccharomyces parasitaris* was described by Trabut ('91) from the grasshopper *Acridium perigrinum* upon which it is a parasite. The yeast *Coccidiascus Legeri* was described by Chatton ('13) as an active parasite in *Drosophila funebris*. *Cryptococcus Anobii* was discovered by Escherich ('00) in the cells of the intestinal wall of the larva of *Anobium paniceum*. *Saccharomyces apiculatus parasiticus* has been described by Lindner ('95) from the body of *Aspidiotus nerii*, and a very similar species causes a fatal disease among caterpillars. *Saccharomycopsis guttulatus* has been studied by Buscalioni ('96) and Wilhelmi ('98). It seems to live as a true parasite in the intestinal canal of birds, reptiles, and mammals. These cases may serve to give a general conception of the pathogenicity of yeasts and yeast-like fungi in relation to animals. A somewhat extended review of the earlier work in this field is to be found in a paper by Vuillemin ('01).

Peglion ('97) was the first to report a parasitic yeast on plants. He described a yeast from diseased hazel nuts in Italy, and created the genus *Nematospora*, species *Coryli*, for it. Schneider ('16) described a second species of this genus, *Nematospora Lycopersici*, from diseased tomato fruits in California.

Nowell ('15) isolated four fungi from diseased cotton bolls in the British West Indies during the seasons of 1915 and 1916. These fungi were described by Nowell ('17), and designated as *Species A*, *B*, *C*, and *D*. He considered them as closely related forms and states that *Species C* and *D* are clearly related to the *Nematospora* species described by Peglion and Schneider. According to Nowell ('17), M. S. F. Ashby, sometime microbiologist in the Jamaica Department of Agriculture, independently discovered *Species A* and *B* in cotton bolls in Jamaica; and Bartlett ('07), Government botanist in British Guiana, obtained *Species A* from cotton bolls, and probably saw the spores of *Species C* or *D* in that country in 1907.

Eremothecium Cymbalariae, a fungus considered by Nowell as agreeing exactly with his *Species B*, was described by Borzi ('88) in Italy as a parasite on the capsules of *Linaria Cymbalaria*. This fungus was redescribed by Arnaud ('13) in France as a parasite on the fruits of *Cachrys laevigata*. It should be noted at this point that Arnaud described and figured two types of fruiting bodies and spores from his cultures. His macrosporangia and macrospores closely resemble the asci and ascospores of Peglion's *Nematospora Coryli*. He no doubt had a mixed culture.

Nematospora Phaseoli was discovered by the writer ('22) in diseased Lima beans from eastern Virginia in 1921, and since that time it has been found to occur more or less generally in fourteen Virginia counties. It has been found once in Lima beans from Tennessee and several times in Lima beans from Alabama. Anderson ('24) also found this species in Lima beans in Illinois in 1923. The frequency, therefore, with which this species is found in Virginia and Alabama indicates that it is of rather general occurrence in those states; and it is believed that a more careful survey will reveal its presence in other Southern States.

The several species of *Nematospora* and *Nematospora*-like fungi have now been found in the fruits of a considerable variety of plants. The hosts as reported thus far are shown in Table 1.

The leguminous plants are predominant in the list, and with the exception of cotton and tomato are the most important from an economic standpoint. My studies indicate that the small Lima or Sieva bean is the most susceptible host for *Nematospora Phaseoli*. The larger types of Lima beans are susceptible but,

as a rule, the infection is less severe. Infection of Bird-eye beans, Black-eye cowpeas and sweet potato has been observed but once. It should be noted, nevertheless, that the Bird-eye bean and cowpea seed showed a high percentage of typical infection and the sweet potato showed an abundant growth of the yeast. No difficulty was experienced in isolating *Nematospora* from these specimens. In pure culture these forms appear to be identical with that from the Lima bean.

TABLE 1.—HOST PLANTS OF *Nematospora* AND *Nematospora*-LIKE FUNGI

Host	*Pathogen*
Corylus avellana (Hazel bush)	N. Coryli Peglion
Lycopersicum esculentum (Tomato)	N. Lycopersici Schneider
Lycopersicum esculentum (Tomato)	Species A, B, D, Nowell
Phaseolus lunatus (Lima bean)	N. Phaseoli Wingard
Phaseolus lunatus (Lima bean)	Species D, Nowell
Phaseolus vulgaris (French bean)	Species D, Nowell
Phaseolus vulgaris (Bird-eye bean)	N. Phaseoli Wingard
Ipomoea Batatas (Sweet potato)	N. Phaseoli Wingard
Vigna catjang (Rounceval cowpea)	Species A, D, Nowell
Vigna sinensis (Black-eye cowpea)	N. Phaseoli Wingard
Dolichos Lablab (Bonavist)	Species D, Nowell
Canavalia gladiata (Sword bean)	Species D, Nowell
Crotalaria retusa	Species D, Nowell
Crotalaria juncea (Sunn hemp)	Species D, Nowell
Tephrosia spp.	Species D, Nowell
Indigofera spp.	Species D, Nowell
Cassia spp.	Species D, Nowell
Gossypium spp. (Annual & perennial cottons)	Species A, B, C, D, Nowell
Ricinus communis (Castor oil bean)	Species D, Nowell
Jatropha urens	Species D, Nowell
Momordica charantia (Coolie pepper)	Species D, Nowell
Asclepias curassavica	Species D, Nowell
Citrus sinensis (Sweet orange)	Species D, Nowell
Linaria Cymbalaria	Eremothecium Cymbalariae Borzi
Cachrys laevigata	Eremothecium Cymbalariae Borzi
Cachrys laevigata	N. Coryli Peglion (?)

SEVERITY OF INFECTION AND ECONOMIC IMPORTANCE

As shown in the table above, the fungi of the *Nematospora* type are capable of infecting the fruits of a wide range of plants. A brief summary of the reports given by various authors shows

the economic importance of these diseases. Peglion, for instance, states that he examined a large number of hazel nuts in Italy during the seasons of 1897 and 1901 and a fairly high percentage of spoiled nuts was found. He reports one case in which 25 per cent of the nuts were spoiled. Schneider had no opportunity to determine the extent of injury on tomato fruits because he was uncertain as to the source of the affected fruits. Nowell, on the other hand, finds *Nematospora* infection fairly common on tomatoes in the British West Indies. There is evidence, therefore, that infection is not uncommon on tomatoes in the tropics. The results of Nowell's studies show also that the *Nematospora* diseases are of considerable economic importance on the fruits of many other plants in the British West Indies. The losses in the case of cotton often run as high as 20 per cent in the early picking season and increase to almost 100 per cent for the late picking season. Nowell states, however, that the prevalence of infection is much greater in some islands than in others, and much greater in some seasons than in others.

My observations have been limited to cases of infection of Lima beans (*Phaseolus lunatus*), Bird-eye beans (*Phaseolus vulgaris*), Black-eye cowpeas (*Vigna sinensis*), and sweet potato (*Ipomoea Batatas*). Of these hosts, the small Lima or Sieva bean is the most severely affected. Infection of the large seeded varieties of Lima beans is, as a rule, slight as compared with that of the Sieva type with small seed. Cases of infection range from those in which only small spots are produced to those extreme cases in which the entire seed is involved. In York County, Virginia, where the disease is most common, several growers have experienced such heavy losses that they no longer attempt to grow the Sieva bean. Losses have been found to range from a trace to 90 per cent of the crop, but, as a rule, they do not exceed 10 to 20 per cent. Although infection is slight during the early part of the season, it becomes very severe during August and September.

My observations of infection on the other hosts named above have been too limited to justify any definite estimate of the losses sustained, but the cases observed indicate that these hosts are subject to severe injury. It seems that the undergroundn habitat of the sweet potato should protect it against inf ecti o

in the field, but *Nematospora* may prove capable of producing a storage rot. Its luxuriant growth on sliced sweet potato suggests this as a possibility, but whether this actually is the case remains at present only a matter of conjecture. When one takes into consideration the wide range of its distribution and the large number of hosts, together with the severity of infection, *Nematospora* must be recognized as a parasite of considerable economic importance.

Infection studies

Peglion obtained no decisive results in inoculating the mature cotyledons of hazel nuts with *Nematospora Coryli*. Schneider apparently made no inoculations on tomato fruits with *Nematospora Lycopersici*. Nowell made a number of inoculations on green cotton bolls with *Nematospora* (*Species D*), and succeeded in getting infection when the bolls were punctured with a needle which had been previously dipped into an agar culture of the organism.

Infection with *Nematospora Phaseoli* has been obtained repeatedly on Lima beans of the small Sieva type under both greenhouse and field conditions. Atomizing the young pods with an aqueous suspension of the organism failed to produce infection. Negative results were also obtained when uninjured pods were smeared with a pure culture of the organism; but infection was readily produced in the seed when the pods were punctured with a fine needle, previously dipped into a pure culture of the yeast. The pods were carefully washed with a 1 to 1000 bichloride solution and allowed to dry before being punctured. Inoculations made in this manner resulted in infections that closely resemble those that occur under natural conditions. The characteristic symptoms were evident within two to three days after inoculation, and were quite conspicuous within seven to ten days.

Similar inoculations failed to produce infection of garden beans (*Phaseolus vulgaris*). Tomato fruits growing in the greenhouse were also inoculated but no infection occurred. It was found, however, that ascospores were formed in the fruits that were allowed to become thoroughly ripe. The sliced ripe fruits make a fairly good medium for the growth of the organism. Inoculations were made on another series of green tomato fruits

growing in the greenhouse. Three species of *Nematospora* were employed in this experiment; namely, *N. Phaseoli* from Lima bean, *N. Sp. D*, Nowell, from cotton, and *N. Sp. D*, Nowell, from tomato. Eighteen fruits were inoculated, six with each culture; and each fruit was punctured at six different points, making a total of thirty-six inoculations with each species.

These fruits were examined as soon as they were thoroughly ripe, but the organism was found in only one of them. This proved to be one of those that had been inoculated with *Sp. D*, Nowell, from tomato. The puncture had healed over, but in a small area near the surface of the fruit the organism was still present. The other punctures appeared the same as those in the controls. The temperature, which is no doubt an important factor in infection, was lower in the greenhouse than it usually is in the field during the summer months and this probably had some influence on the results obtained.

THE RÔLE OF INSECTS IN INFECTION

Peglion states that *Nematospora* lesions on young hazel nuts are mostly near the micropyle, and expresses the opinion that the parasite enters by way of the style before the pericarp becomes woody. This may be true but the type of lesion described by him on the cotyledons of the hazel nut strongly suggests insect punctures as the portal of entry. Nowell (**'17**, **'20**) and his associates have found that *Nematospora* infection in cotton bolls and the fruits of many other plants is accomplished only by the punctures of certain insects; namely, the cotton stainer (*Dysdercus delauneyi*), the green bug (*Nezara viridula*), the leaf-footed tomato bug (*Leptoglossus balteatus*), and the red tomato bug (*Phthia picta*). The first two are the most important.

Natural infection of Lima beans and cowpeas with *Nematospora Phaseoli* in my experience is apparently entirely dependent upon the puncture of the green bug (*Nezara hilaris**). This insect is always present wherever infection occurs, and the lesions on the cotyledons always develop around its punctures. The severity of infection depends directly upon the number of insects present; and this fact, together with high temperature,

* Specimens of this insect were identified by Dr. Herbert Osborn of Ohio State University in 1923.

explains why the percentage of infection is so high during the latter part of the season.

Nezara viridula, an insect almost indistinguishable from *Nezara hilaris*, feeds upon pecans in the Southern States and produces a disease known as kernel-spot. The lesions of this disease are so similar to those of *Nematospora* infections on the fruits of other plants that the writer at one time was of the opinion that perhaps *Nematospora* was associated with the kernel-spot disease. The fact, too, that kernel-spot is most severe in groves where cowpeas are grown as a cover crop supports this view. With these facts in mind, several lots of affected pecans from Georgia were examined but *Nematospora* was not found. Platings were also made from some of the most typical lesions, but *Nematospora* failed to develop.

Insects as carriers of infection

The cotton stainer (*Dysdercus delauneyi*), the green bugs (*Nezara viridula* and *Nezara hilaris*), the leaf-footed tomato bug (*Leptoglossus balteatus*), and the red tomato bug (*Phthia picta*) are so closely associated with *Nematospora* infection on the various hosts that they appear to be carriers of infection. To test this point, Nowell collected *Dysdercus delauneyi*, *Nezara viridula*, *Leptoglossus balteatus* and *Phthia picta* from fields where *Nematospora* infection was common on cotton and placed them on caged plants with healthy bolls. The punctures made by these insects into the caged bolls resulted in typical *Nematospora* infection in the majority of cases, and an examination revealed the presence of the organism. According to Nowell, *Edessa meditabunda* also feeds on cotton bolls and the fruits of many other plants in the British West Indies, but he has never succeeded in getting infection from its punctures on caged plants.

I have conducted similar experiments with *Nezara hilaris*. Specimens of this insect were collected from gardens where *Nematospora* infection was present on Lima beans and were transferred to another section and placed on caged plants with disease-free pods. The pods on these plants when examined two weeks later showed numerous punctures and the punctured seed showed typical infection. The organism was readily found in the lesions around the punctures.

These results indicate that *Nematospora* is carried by the

above named insects, but whether it is carried internally or externally remains to be determined. Nowell has attempted to isolate it from *Dysdercus delauneyi* and *Nezara viridula* but without success, and I have had the same experience with *Nezara hilaris*. Other yeasts and yeast-like fungi are very readily obtained from this insect, but I have consistently failed to isolate *Nematospora Phaseoli*. I am inclined, nevertheless, to believe that these insects are themselves infected with the organism. The negative results obtained thus far are very probably due to improper technique.

The relation of host to parasite

Infection is apparently restricted to the fruits of the several hosts of *Nematospora*, not a single case being reported as yet where any other portion of the plant is affected. This suggests that infection is dependent upon the presence of an abundant supply of available carbohydrates, such as is found in fruits and in the fleshy cotyledons of certain seeds.

My studies of *Nematospora Phaseoli* have shown that infection may occur at any time during the development of the Lima bean seed, but in order to cause much injury it must take place before the seed is half grown. In case of early infection the seed may either be killed outright or only severely stunted in its development. If infection occurs near maturity only small lesions are produced on the cotyledons and the seed is not materially injured. In the majority of cases the testa remains intact and the affected region is wrinkled, somewhat sunken and of a dark-brown color. In rare cases the testa is ruptured at the point of infection and a crater-like lesion is produced on the surface of the cotyledon. The affected tissues are collapsed, grayish-brown in color and granular in texture. The cells in the affected region apparently are low in starch content.

Cytology of the yeasts

Although our knowledge of the cytology of the yeasts has been greatly advanced during the last quarter of a century, it is still on a rather unsettled basis. The much discussed problem as to whether or not yeast cells possess a typical nucleus has finally been answered in the affirmative, but beyond this point our knowledge is quite vague. There is, however, considerable

divergence in this respect between the widely different groups now included under the general conception of the yeasts. Of these groups, the cytology of that of *Schizosaccharomyces* is most completely understood.

The exact method of nuclear division may be said to be a matter of dispute for all yeasts. Some investigators contend that it is always amitotic; others are of the opinion that it is amitotic at the time of budding and mitotic in the ascus during ascospore formation; while still others contend that it is mitotic for both processes. Furthermore, such points as the type of spindle, presence or absence of astral rays, centrosomes, chromosomes, etc., for most yeasts remain as yet practically untouched. Then, too, our knowledge of the nuclear phenomena involved in the origin of the ascus and the manner of ascospore formation cannot be regarded as settled yet for any single yeast.

Presence of a nucleus

Following the discovery of the cell nucleus by Robert Brown in 1831 and Meyen in 1826, and the development of the conception of protoplasm by Von Mohl in 1846, and others, the cellular make-up of the common brewers' yeast (*Saccharomyces Cerevisiae*) was an object of considerable study, particularly as to whether its cells have a nucleus. We can recognize in this connection two groups of observers: (1) those who, like Nägeli ('44), Schleiden ('49), Schmitz ('79), Strasburger ('84, p. 158, *f.* 77), Zalewski ('86), Hansen ('86), Zacharias ('87), Moeller ('92, '93), Janssens and Le Blanc ('98), Dangeard ('93, '94), Buscalioni ('96), Maffucci and Sirleo ('95), Hofmeister ('00), Bouin ('98), Errera ('98), and Hirschbruch ('02), reported as nuclei granules or bodies in various positions in the yeast cell; and (2) those who, like Krasser ('85), Hieronymus ('93), Stecksén ('01), and Wager and Peniston ('10), reported chromatic granules or other aberrant structures as substitutes for the nucleus. Brücke ('98), Raum ('91), and Macallum ('95) denied the existence of nuclei in the cells of brewers' yeast. The more recent studies of Guilliermond ('02), Swellengrebel ('05), Fuhrmann ('06), and others have shown that the yeast nucleus in all of its general characteristics is comparable to that of the higher fungi.

DIVISION OF THE NUCLEUS

On the question as to whether the reproduction of the yeast nucleus is by direct or indirect division, we can again distinguish, as noted above, several classes of contentions in the literature: (1) those of Dangeard, Hofmeister and others, who hold that nuclear division is direct at the time of budding; (2) those of Janssens and Le Blanc, and Bouin, who hold that it may be either direct or indirect for budding, but is always indirect for ascospore formation; (3) those of Swellengrebel, and Fuhrmann, who hold that it is indirect for budding; (4) those of Hirschbruch, who holds that it is indirect for both budding and ascospore formation; (5) those of Wager and Peniston, and Kohl ('07), who hold that it is direct in both budding and ascospore formation; and (6) those of Guilliermond, and Buscalioni, who contend that it is direct for budding and indirect for ascospore formation.

EXISTENCE AND NUMBER OF CHROMOSOMES

Wager ('98) studied nuclear division in the asci of *Saccharomyces Cerevisiae*, *S. Ludwigii*, and *S. pastorianus*, and observed deeply stained granules which he was inclined to call chromosomes; but in 1910 he and Peniston, after repeating these studies, make the following statement: "We are not now inclined to lay any stress upon the suggestion (Wager, 1898) that the chromatic granules are chromosomes. The whole process appears to be one of direct division or fragmentation, in which nothing in the nature of a spindle figure or definite chromosomes can be observed."

Swellengrebel ('05) studying the nuclei of compressed yeast cells (*S. Cerevisiae*) at the time of budding, gives the chromosome number as four. He describes what he calls an achromatic spindle and what he considered to be centrosomes. The four chromosomes arrange themselves on the equator in the form of a ring while the linin is being transformed into the spindle. The chromosomes divide and four pass to each pole of the spindle where the two daughter nuclei are reconstructed. Fuhrmann ('06) a few months later, confirmed Swellengrebel's results. He studied nuclear division in the budding cell of *S. ellipsoideus*. He reports the existence of an achromatic spindle with a centrosome at each pole. He also gives the chromosome number as

four, and states that these divide, giving four daughter chromosomes for each of the daughter nuclei.

Guilliermond ('17) reported the results of his studies on the nuclear division in the ascus of *Schizosaccharomyces octosporus*. He states that this species is much more favorable for cytological study than other yeasts. He was able to demonstrate chromosomes and an achromatic spindle with centrosomes but found the chromosomes too small to permit of an accurate count; nevertheless, he figures about four or five passing to each pole of the spindle during the late anaphase. He states that his studies on *S. Cerevisiae* and *S. ellipsoideus* have demonstrated that the pretended mitotic figures described by Swellengrebel and by Fuhrmann resulted from an erroneous interpretation of the vacuole and granular material contained in it, or in the cytoplasm.

Conjugation and ascus formation

Schiønning ('95) described the ascus of *Schizosaccharomyces octosporus* as resulting from the conjugation of two sister cells, and Hofmeister ('00) described a nuclear fusion in the conjugating cells. Guilliermond ('01) confirmed Hofmeister's observations. He described the ascus of *S. octosporus* as arising from the conjugation of two identical cells lying side by side in the same colony. The cells become united by a conjugation canal formed by the fusion of two outgrowths from the gametic pair. The wall separating the tips of the tubes is dissolved and the two nuclei of the conjugating cells migrate into the conjugation canal and fuse to form the primary nucleus of the ascus. The ascus increases in volume while its nucleus undergoes two, or sometimes three, successive karyokinetic divisions, which gives four or eight nuclei for the ascospores. Guilliermond found also that the ascus in this species sometimes originates from a single cell. In that event, two cells become united by a conjugation canal but the separating wall remains intact, and each cell develops into an ascus.

Guilliermond ('02) showed that the asci of *Schizosaccharomyces pombe* and *S. Mellacei* arise in the same manner as described for *S. octosporus*. In these species, however, the asci more frequently arise from single cells. It may be the result of the failure of the separating wall between the two cells to be

reabsorbed, or a single cell may develop into an ascus without even attempting to conjugate with another. Šulc ('10) described a conjugation in the ascus formation of *S. Aphalarae calthae* which appears to be analogous to that of *S. octosporus*. Nakazawa ('14) is reported by Guillermond and Tanner ('20) as having described a similar process in the ascus formation of *S. santawensis* and *S. formosensis*.

An isogamic conjugation is also known to occur in the ascus formation of the budding yeasts, especially in the genus *Zygosaccharomyces*. Barker ('01) described it for *Z. Barkeri*, and Pearce and Barker ('08) described it for *Zygosaccharomyces F.* An analogous process has been described for *Z. priorianus* and *Z. mandshuricus* by Klöcker ('05), for *Z. javanicus* by de Kruyff ('08) for *Z. japonicus* by Saito ('09), for *Z. lactis* by Dombroski ('10), and for *Z. major* by Takahashi and Yukawa ('12). Certain of these authors state that asci may also arise from single cells in the species which they studied.

An isogamic conjugation has also been described in the ascus formation of certain other genera. Chatton ('13) for example, described it for the yeast *Coccidiascus Legeri*, and Schneider ('16) reported it for *Nematospora Lycopersici*. Schneider, however, states that there is evidence that the asci of *N. Lycopersici* may develop from a single cell.

In addition to the type of conjugation described above, certain authors have described processes which they consider to be heterogamic. Pearce and Barker ('08) described a yeast which they designated as *yeast G* in which the conjugation tends towards heterogamy. Guilliermond ('11) described what he considers to be a strictly heterogamic process in the yeast *Zygosaccharomyces Chevalieri*. The asci in this species result from the conjugation of two cells of different dimensions. The contents of the small cell pass into the large one through the conjugation canal. The large cell separates itself from the small one by means of a wall, and later develops into the ascus. Guilliermond ('18) described the same type of conjugation for *Z. Nadsonii*, and in a paper of the following year ('19) for *Z. Pastori*. In these species conjugation occurs between a mother cell and an incompletely developed daughter cell, the mother cell in this case playing the part of the female gamete, while the daughter cell functions as the male.

Klöcker ('06) found that the conjugation in *Z. priorianus* in some cases was isogamic but in others it was heterogamic, occurring between a mother cell and a bud still attached. He also found that many cells formed ascospores without undergoing conjugation. Guilliermond ('11) described the same types of conjugation for *Debaryomyces globosus*, and Konokotina ('13) for *D. tyrocola*. Nadson and Konokotine ('12) found that the conjugation in the species *Nadsonia fulvescens* and *Nadsonia elongata* always takes place between a mother cell and a bud formed by it. The contents of the bud pass into the mother cell, which, instead of developing directly into an ascus, gives birth, by budding, to a new cell into which its contents are poured; and this cell develops into an ascus.

In certain yeasts such as *S. Ludwigii*, *Willia Saturnus* and yeast *Johannisberg 11* the asci develop from single cells, but the ascospores as a rule conjugate two by two at the time of germination. This phenomenon was first described by Hansen ('91) in *S. Ludwigii*. The ascospores become united by means of a conjugation canal into which the nucleus and cytoplasm of each pass, and in which nuclear fusion occurs. A zygospore is formed which later germinates by sending out a germ tube from which numerous vegetative cells are produced by budding. About one-fourth of the ascospores of this species germinate without any sign of conjugation. Guilliermond ('03) found that the conjugating ascospores in this species as well as in *Willia Saturnus* often germinate into asci instead of producing vegetative cells. The single ascospores were found to do the same thing. Hansen ('91) showed that the ascospores of *S. Cerevisiae* and "*Johannisberg II*" would also, under certain conditions, germinate directly into asci either with or without conjugating. Marchand ('13) described a similar conjugation of ascospores in *S. intermedius*, *S. turbidans*, *S. validus*, *S. ellipsoideus*, *S. Vini Muntzii*, *S. Willianus*, *S. Bayanus*, and "*Johannisberg I.*" Guilliermond ('17b) described a yeast from pulque in which the asci develop from single cells. The ascospores conjugate two by two at the time of germination and in unfavorable solid culture media change directly into asci. This species, like *S. Ludwigii* and *Pichia membranaefaciens*, often forms a mycelium on the surface of old cultures. In that case, each cell in the mycelium may develop into an ascus with the normal number of ascospores.

NUMBER OF ASCOSPORES

The number of ascospores contained in the ascus of various yeasts runs from one to eight. The number is usually fixed in any one species or at least a certain number is found to predominate. In *Monospora bicuspidata*, *Nadsonia fulvescens*, *Nadsonia elongata*, *Debaryomyces tyrocola* and others, a single ascospore is produced. In *Schwanniomyces occidentalis*, *Debaryomyces golobosus*, *Zygosaccharomyces Nadsonia*, one to two ascospores are produced, with one predominating. The ascus of *Z. bisporus* contains two ascospores as a rule. In *Saccharomyces Cerevisiae* the number varies between one and five, but the number four is more frequent. In *S. pastorianus* the same variation in number obtains but the number two predominates. In *S. Ludwigii* and the yeast *Johannisberg II*, the number is almost always four. In *Schizosaccharomyces octosporus* the number is either four or eight. In *S. Mellacei* and *S. pombe* the number is invariably four. In *Nematospora Coryli*, *N. Lycopersici*, and *Coccidiascus Legeri*, the number is almost always eight. The variation in number of ascospores within a genus is apparently very little greater than that of a single species.

METHOD OF ASCOSPORE FORMATION

As previously noted, the real nature of ascospore formation in the yeasts is none too well understood. Are the yeast ascospores cut out by free cell formation, or are they formed by cleavage? Wager ('98) describes the spores of *S. Cerevisiae* and *S. Ludwigii* as lying free in the protoplasm of the ascus. The spores are said to be at first very small, but they gradually increase in size at the expense of the surrounding protoplasm, a thick cell-wall being produced around each, until finally they completely fill the mother-cell.

Janssens and Le Blanc ('98), describing the process in the same species, write: "Pour compléter les spores, les noyaux s'entourent d'une partie plus dense de protoplasme, autour de laquelle une membrane se forme librement sans la participation de la membrane de la cellule-mère. L'apparition de cette membrane peut surtout s'étudier, quand il ne se forme que deux spores, mais on peut encore en poursuivre tous les détails, dans des cas de très bonne fixation, sur des cellules produisant quartre

spores. Il se forme une sorte de plaque cellulaire librement dans le protoplasme à une certaine distance du nucléole. Les trabécules du protoplasme se régularisent et bientôt, par le dépôt d'une substance squelettique analogue à la cellulose, on voit apparaître dans le protoplasme une zone circulaire plus réfringente entourant un protoplasme très dense."

Guilliermond ('02) has studied ascospore formation in a large number of yeasts, and has described the presence of spore plasm and epiplasm as in the asci of the higher Ascomycetes. He is of the opinion, too, that the ascospores are formed by free cell formation, but he states that the figures are too small to allow one to follow the details of the process.

Cytology of Nematospora Phaseoli

The material used in this study was grown in pure culture on either beer wort agar or sweet potato slants. Cultures ranging from one to ten days old were used in order to obtain all of the different types of cells and stages in the development of the organism. Cultures one to three days old were found to supply excellent material for the study of the development of the asci and ascospores; those three to five days old were very good for ascospore germination; and those from five to ten days old supplied excellent material for the study of the direct germination of the ascospores into asci, the conjugation of ascospores, and the formation of asci in the mycelial branches.

Numerous solutions were tested for killing and fixing the material; and, of those tested, Flemming's weak solution, diluted 1 to 200 with distilled water, was found to give the best results. This solution as a rule gave good fixation with little or no shrinkage. A small quantity of the culture material was transferred to about ten to fifteen drops of the fixing solution by means of a platinum loop. After the material had remained in the fixing solution for 15 to 30 minutes, it was mounted for staining by the stipple method, as described by Harper ('99) and others, for mounting germinating smut spores. The ether-celloidin method, as described by Davis ('22) for mounting germinating spores, was also used with very good success, especially when the material was stained with haematoxylin.

Several stains, including the majority of those recommended by the various authors who have studied the yeasts, were tested;

but Flemming's triple stain was the only one that gave satisfactory results. Haidenhain's iron-alum-haematoxylin was found to be very good for staining ascospores and resting nuclei, but otherwise it failed to bring out the details that are shown with the triple stain.

ASCOSPORES AND THEIR GERMINATION

The mature ascospores are comparatively very long and slender and consist of two cells of about equal length, with the basal one ending in a long whip-like appendage. When stained, the basal cell exhibits an alveolar cytoplasm and a single well defined nucleus, which consists of a deeply stained nucleole, a less densely stained chromatic reticulum, nuclear sap, and a nuclear membrane (FIGS. 18a, 18b). The nucleus is situated in the upper end of the cell near the septum which separates the ascospore into two cells, and tends to be slightly pointed in the region where the nucleole is located. The apical cell of the ascospore shows quite a different aspect. The cytoplasm in this cell, which apparently begins to disintegrate very early, appears in the form of densely stained clumps, and no nucleus can be distinguished.

At the time of germination, the upper region of the basal cell of the ascospore swells and becomes spherical in shape just below the transverse septum, the nucleus enlarges and divides to form nuclei for the cells that later bud off from this region. The nuclei at this stage are very small, about 2 microns in diameter; but I have observed in a very few cases a very minute spindle-shaped figure which appears to be similar to the undoubted mitotic figures that are found later in the ascus. The nucleus may divide either before or after budding is evident. In some cases it divides and two nuclei are found before budding begins (FIG. 17); in other cases a bud cell is well formed before nuclear division takes place (FIG. 15a). In still other cases, which seem rare, one large nucleus and two small ones are seen in the swollen region before budding occurs (FIG. 12), indicating perhaps that the division of the mother nucleus has been followed by the division of one of the daughter nuclei. The apical cell has never been observed to germinate; its contents undergo further disintegration and it remains as an apparently functionless appendage. The question as to its nucleus is discussed

below. From one to six chains of bud cells may be seen attached to the swollen region of the ascospore. These cells later become dissociated and may either bud as yeasts or develop into asci. The yeast cells contain a single nucleus (FIG. 19) which resembles that of the ascospore. Nuclear division has not been observed in these cells during budding.

In addition to the method as described above, the ascospore often exhibits several other modes of germination, the cause of which has not been definitely determined. I am inclined to believe that the budding type of germination as described above is the most common when fresh solid media are used, although it may occur under other conditions.

The second type of germination, which is characterized by the production of one or more germ tubes from the swollen region of the ascospore, occurs in liquids and also in old cultures on solid media. However, a sharp line of demarcation cannot be drawn between the conditions that are necessary for this and the first mode of germination. Two ascospores lying side by side in apparently the same environment may exhibit different types of germination; one by budding, and the other by germ-tube production. Furthermore, ascospores may be observed with a chain of bud cells attached to one side and a septate mycelial hypha extending from the other side. Then, too, the method of germination may be intermediate between budding and the production of germ tubes.

In the third type of germination the basal cell of the ascospore swells and develops directly into an ascus (FIG. 20). This occurs in old cultures on solid media after an abundant growth has been made. Whether this is due to toxic substances or to a lack of food, or both, has not been determined. We should note here the cases in which two adjacent ascospores produce conjugation tubes which fuse at their tips and develop into an ascus. This procedure seems to be rather unusual, and yet it has been observed several times in old cultures, especially on Irish and sweet potato slants. The drying out of the medium may also be an important factor in determining the type of ascospore germination.

NUCLEAR PHENOMENA INVOLVED IN ASCUS FORMATION

As previously stated, the cells that are produced by budding at the time of ascospore germination become dissociated and either develop individually into asci or multiply further by budding. If budding occurs, the individual cells thus formed may in turn develop into asci. Since this seems to be the most common method of ascus formation, I shall begin by describing the nuclear phenomena involved in this process.

The small uninucleate cell enlarges very rapidly, apparently with a proportionate increase in the size of its nucleus. The elongation of the cell takes place more rapidly than the increase in its cross diameter, thus giving it a cylindrical shape. The nucleus is very distinct and exhibits a densely stained nucleole, nuclear membrane, and a less densely stained chromatic reticulum. The cytoplasm at this stage shows a very regular alveolar structure. The prophases of the first division of the nucleus become evident a little later. The chromatin becomes condensed into densely stained threads which in some cases appear to be attached to a point on the nuclear membrane from which they radiate more or less irregularly into the nuclear cavity (FIGS. 3, 6). In some cases a minute body can be faintly seen at the point of attachment of the chromatin threads on the nuclear membrane. I have been able to observe this orientation of the chromatin only in a few instances, and am in doubt whether these figures correspond entirely to those given by Harper ('05) for the asci of the higher Ascomycetes. A large oval shaped nucleole can be distinctly seen lying in the colorless nuclear sap that surrounds the chromatin strands. It is almost invariably situated near the nuclear membrane. The nucleus at this stage, though much smaller, bears a striking resemblance to the spiremes figured by Harper in the asci of *Phyllactinia* and *Erysiphe*.

Following this stage the first nuclear division occurs, which is usually in the direction of the long axis of the cell (FIG. 2). The spindle appears first as a minute streak extending across the nuclear cavity, with the chromatin and nucleole situated slightly to one side. The spindle is at first strictly intranuclear, but as it elongates the nuclear membrane disappears, and five chromosomes can be fairly accurately counted. The chromosomes are globular in shape and measure about 0.45 microns in diameter,

and yet they are distinct in well differentiated preparations. The nucleole is very distinct at this stage also and is generally situated near the center and slightly to one side of the spindle. It measures about one micron in diameter.

The first division of the nucleus has been found in relatively few cases and I have been unable to follow the later stages. The second and third divisions, however, are very easily found, and in them the process can be fairly accurately observed. The two daughter nuclei resulting from the first division are at first very small and are closely associated. They later pass to the opposite ends of the ascus, enlarge, and prepare for the second division.

The daughter nuclei in the resting stage are identical in appearance with the mother nucleus. A more or less polarized spireme is formed (FIG. 3) which is soon followed by the formation of an intranuclear spindle. Four or five chromosomes can be counted in the equatorial plate stage, and a little later nine or ten can be distinctly seen scattered along the spindle (FIG. 8). Some of these figures show densely stained strands radiating from their poles, which may be interpretated as astral rays. Centrosomes, however, cannot be distinguished with certainty in these figures. The ascus at this stage is about two-thirds mature size. The spindle elongates and becomes very narrow. Four or five chromosomes pass to each pole and the daughter nuclei are formed. The nucleole may persist on the side of the spindle during the entire process of nuclear division.

The four daughter nuclei become distributed at approximately equal distances throughout the length of the ascus. They then divide as a rule in the direction of the long axis of the cell (FIG. 9) and produce eight nuclei for the ascospores. The spindles in the third division are much smaller than in the first and second divisions and the chromosomes appear to be proportionately smaller in size. The chromosome number, however, appears to be the same throughout the three divisions.

The nuclear phenomena in the asci that arise by the direct germination of the ascospores are apparently the same as in the asci that develop from individual bud cells. This type of ascus formation is unusual, in that the upper region of the basal cell of the ascospore simply swells out into a large outgrowth, which assumes the form of a normal ascus. What causes or stimulates

the cell to make this tremendous outgrowth? Does a nucleus come in from the apical cell and fuse with that of the basal cell before this growth occurs? I am not prepared to answer these questions, but the question as to a nuclear fusion will be further discussed below.

The swollen region of the basal cell of the ascospores apparently contains a single nucleus at first (FIG. 20), which later divides in the same way as described above (FIGS. 21, 22). Here again five chromosomes can be counted in the equatorial plate stage. After division is completed, the daughter nuclei separate and undergo a second division (FIG. 24). These divisions may occur either in the direction of the long or short axis of the cell. The same thing holds for the divisions in the first type of ascus, but nuclear division is as a rule in the direction of the long axis of the ascus. The third divisions have not been observed in asci arising directly from ascospores.

The germ-tube type of ascospore germination results in the development of a mycelium in which intercalary asci are often produced. The individual cells of the hyphae develop directly into normal asci (FIG. 31) in which four to eight ascospores are produced (FIGS. 29, 30). These cells are at first apparently uninucleate, but as they increase in size their nuclei increase in number. Nuclear division has not been observed in these asci.

The fourth type of ascus formation follows the conjugation of two germinating ascospores. The basal cell of each of the ascospores swells and sends out a short outgrowth. These outgrowths come in contact and fuse end to end to form the conjugation canal. This conjugation results in the production of an ascus in which four or eight ascospores are formed. The apical cells and the tips of the basal cells of the two ascospores remain attached to the membrane of the mature ascus (FIGS. 32, 33).

The nuclear phenomena in this type of ascus have not been determined, but there is evidence that a nuclear fusion occurs in the tube connecting the conjugating ascospores. Each of the basal cells of the pair of ascospores contains a nucleus before conjugation occurs, whereas only one is found in the connecting tube after conjugation takes place. As the ascus develops, the number of nuclei increases to two, then to four, and finally to eight. Ascospores are then formed. Here again nuclear division figures have not been observed.

Finally, there is evidence that the asci of *Nematospora Phaseoli* may arise from the conjugation of two yeast cells. Although I have fairly good evidence of this in some of my preparations, I do not believe that it is so common as the process by which the ascus arises from a single bud cell. On the other hand, Schneider states that the asci of *N. Lycopersici* most commonly arise from a conjugation of two cells. The same type of conjugation has been described by numerous investigators as occurring among the species of *Zygosaccharomyces* and *Schizosaccharomyces*, and yet all of these investigators find that the asci in these species may also arise from individual cells.

I find stages in my preparations that suggest a conjugation of two cells, the strongest indication being a constriction that approximately divides the ascus into two equal parts. Two nuclei are present, one in each enlargement. Then in larger asci where the constriction is still apparent only one nucleus may be found. In still larger asci, two nuclei again appear. Unfortunately, however, stages appear that do not fit into the above series. Figures are found in these asci that have the appearance of nuclear fusions, but here again one must be skeptical because similar stages are found in the asci that develop from single cells, and also in those that arise directly from the germination of single ascospores. Janssens and Le Blanc described a nuclear fusion in the ascus of *S. Cerevisiae* and regarded it as a sexual fusion. Guilliermond has repeated these studies but he was unable to verify their results. He contends that there is no nuclear fusion in a yeast ascus which arises without preliminary spore or cell conjugation. My results thus far do not justify any definite statement in regard to nuclear fusion in the ascus of *N. Phaseoli*.

Ascospore formation

The ascus is practically full size at the time of the third nuclear division. It is then 70 to 80 microns long and about 10 microns in diameter. The four mitotic figures can be clearly seen lying in the faintly stained alveolar cytoplasm (FIG. 9). The final stages in these division figures and the initial stages in the formation of the ascospores have not been observed with certainty. The ascospores, however, can be distinctly seen very early in their development. They appear as small globular

bodies about 1 to 1.5 microns in diameter, and show a granular cytoplasm. They possess a definite bounding membrane that separates them from the surrounding vacuolated epiplasm. Whether this bounding membrane is formed by the fusion of astral rays as described by Harper ('05) and others in free-cell formation of ascospores in the higher Ascomycetes remains to be determined. On the other hand, there is no doubt as to the existence of an epiplasm distinct from the spore-plasm contained in the young ascospores.

The ascospores at this stage stain so densely that it is very difficult to distinguish their nuclei. The young globular ascospores now develop into the mature two-celled stage in which they are long and slender. They first assemble near the center of the ascus and then begin to elongate by sending out a pointed outgrowth that reminds one of a germ tube (FIG. 14). The four on the left elongate towards the right end of the ascus and the four on the right elongate towards the left end. A single nucleus can be distinguished in some cases when the ascospores are about one-half their mature length. As this elongation proceeds the epiplasm is more or less used up and finally the eight mature two-celled ascospores are formed, filling the entire ascus (FIG. 11). The mature ascospores are arranged in two opposite bundles of four spores each, with their whip-like appendages interlocking or more frequently curved around the opposite bundle.

The basal cell of the mature ascospore contains a single nucleus that is very easily seen, and an alveolar cytoplasm. The apical cell, as noted above, is apparently without a nucleus and its cytoplasm even at a very early stage appears to have undergone disintegration, and consists of densely stained masses (FIG. 11). It is possible that the nucleus of this cell passes into the basal cell at an early stage and fuses with its nucleus, but the evidence for this is lacking at present. I have been able to find only one case in which such an occurrence was suggested. In this case, the ascospores seemed immature but had reached the two-celled stage. The apical cells failed to show a nucleus as usual, but the basal cell of one contained two distinct nuclei. The basal cells of the others contained one nucleus as usual. I could detect no difference in the size of these nuclei.

Observations on ascospore formation in certain of the higher Ascomycetes are of interest in this connection. Faull ('04) has

found that the ascospores of several of the species of *Podospora* present a curious case of the formation of a septum in the spore without a previous division of the nucleus. The young ascospores are at first compressed and spherical but rapidly become cylindrical or rod-like. This is followed by an enlargement of the upper end in which the nucleus is to be found. The nucleus next moves down to the entrance of the small end, a septum is formed, and it passes back to the center of the upper part. The appendage develops no further, and gradually loses its cytoplasm. Brooks ('10), studying ascopore formation in *Gnomonia erythrostoma*, found that after the spore membrane has been laid down the nucleus undergoes division towards the base of the ascospore. One of the two daughter nuclei thus formed passes to the extremity of the spore, and a septum is laid down between the two nuclei, cutting the ascospore into cells of unequal size. The nucleus of this tail-cell gradually disorganizes. He found also in germinating the ascospores, that only the larger cells send out germ tubes.

It should be noted, however, in this connection that the sterile cells in the ascospores of these fungi are much smaller than the functional cells; whereas in *N. Phaseoli* the apparently functionless cell is equal in size to the functional cell.

Discussion

My cytological studies have shown that the ascus of *Nematospora* is typical in respect to the differentiation of spore-plasm and epiplasm. The ascus is uninucleate at a rather young stage, and this single nucleus undergoes the regular triple division characteristic of practically all asci. Eight ascospores are regularly formed, though I have observed a few cases in old cultures where a smaller number was formed. It could be clearly seen in these cases that the reduced number was due to the abortion of some of the young spores, as in the case of the mildews such as *Phyllactinia* and *Erysiphe*.

The ascospores are unquestionably formed by free cell formation: that is, they are cut out of the ascus as globular cells surrounded by the epiplasm which is later more or less used up in their development to mature size. The figures, however, are too minute to permit one to follow the exact details of the process of spore delimitation. I have also so far failed to settle the

further point as to the existence of a nuclear fusion in the young ascus.

There seems no adequate reason for regarding *Nematospora* as a "doubtful Ascomycete," so far as its ascus is concerned. As noted earlier, Guilliermond ('12) puts *Nematospora*, *Monospora*, and *Coccidiascus* together as his fifth group of *Saccharomycetes*, and designates them as budding yeasts with uncertain affinities. As recognized by Guilliermond, the relationship of this group to the other groups is by no means clear. Guilliermond's series consists of the following groups: (1) Yeasts multiplying by partition; asci usually derived from a conjugation. (2) Budding yeasts; asci usually derived from a conjugation. (3) Budding yeasts which form a slimy scum in sugar solutions; ascospores round or oval in shape. (4) Budding yeasts which generally produce ether; ascospores characteristically hat-shaped. (5) Yeasts in which the relationships are not well known.

In my opinion *Nematospora* has a typical ascus, and at the same time is unquestionably a member of the yeast series. The evidence of the relationship of the whole series to the Ascomycetes is thus strengthened. Whether *Nematospora* is to be regarded as a primitive or degenerate type is a more difficult question. The whole group with its interesting relations to the insects and incipient parasitic habits on green plants appears to be excellent material for the study of the general problems as to the origin and relationship of the entire yeast series.

The genus *Nematospora* is apparently closely related to the genera *Monospora* and *Coccidiascus* which it resembles morphologically, especially in the shape of the ascospores and in the production of budding yeast-like cells. *Monospora* differs from the others in having a single ascospore. This genus should be studied cytologically to determine whether there is a triple nuclear division in the ascus. These three genera in my opinion constitute a phylogenetic unit.

The genera *Eremothecium* Borzi, and *Protascus* Dangeard, somewhat resemble *Nematospora* in respect to the shape and arrangement of their spores. *Eremothecium* has about 50 spores formed in its fruiting body and there is no evidence that they are formed by free cell formation. *Protascus*, on the other hand, has only 32 spores, and Dangeard has presented evidence that shows that they are formed by free cell formation. Asci

with more than eight spores are of course well known, for example, *Rhyparobius*, *Podospora*, *Thecotheus*, etc. But whether the asci of *Eremothecium* and *Protascus* belong to this class must be determined by further cytological study. The further point as to whether the resemblance of *Eremothecium* and *Protascus* should be regarded as merely accidental or as an indication of relationship remains unsettled as yet.

The species of the genera *Nematospora*, *Monospora* and *Coccidiascus* are all parasites. *Monospora bicuspidata* is parasitic on *Daphnia*, *Coccidiascus Legeri* is parasitic on *Drosophila funebris*, and the five species of *Nematospora* are parasitic on the fruits of a wide range of plant families. *Nematospora species C* and *D*, Nowell, are parasitic on the fruits of the following plants in the British West Indies; tomato, Lima bean, sword bean, castor oil bean, cowpea, cotton, coolie pepper, sweet orange, *Asclepias* sp., *Jatropha* sp., *Cassia* sp., *Indigofera* sp., *Tephrosia* sp., *Crotalaria* sp., and *Dolichos Lablab*. Infection, according to Nowell, is brought about by the punctures of the cotton stainer (*Dysdercus delauneyi*), the green bug (*Nezara viridula*), the leaf-footed tomato bug (*Leptoglossus balteatus*), and the red tomato bug (*Phthia picta*).

Nematospora Coryli is parasitic on hazel nuts, *N. Lycopersici* is parasitic on tomato fruits, and *N. Phaseoli* is parasitic on Lima beans, Bird-eye beans, and Black-eye cowpeas. Infection with *N. Phaseoli*, as I have shown, takes place in the punctures made by the green bug (*Nezara hilaris*). We have then two genera of the group, *Monospora* and *Coccidiascus*, passing their whole life history as parasites in respectively a crustacean and a fly, *Daphnia* and *Drosophila*, while the third genus, *Nematospora*, with its much larger number of species, passes its complete cycle of development, so far as yet known, as a parasite in some one of the wide range of host plants just enumerated, but again, so far as known, depends entirely on insects, such as *Dysdercus delauneyi*, *Nezara viridula*, *Nezara hilaris*, *Leptoglossus balteatus* and *Phthia picta*, for gaining access to its hosts. The situation is most interesting from the standpoint of the whole question of insect carriers of pathogenic organisms.

The method of infection of *Drosophila* is unknown, though, as Chatton notes, the flies feed on mother of vinegar. Metchnikoff does not state the method of infection in *Daphnia*, but he reports

large quantities of vegetative cells, asci and ascospores in the tissues of the sick and dead *Daphnia*. He also reports the presence of free ascospores in the intestinal tract of the healthy *Daphnia*. This naturally suggests that the ascospores are taken into the intestinal tract by the feeding of *Daphnia* in contaminated water.

The question whether *Nezara hilaris* is also a host for *Nematospora Phaseoli*, and may possibly carry it through the winter is a matter of obvious importance. Infection with *Nematospora Coryli* and *N. Lycopersici* has not been reported as due to insect punctures, but this is probably due to the fact that the insect punctures were overlooked. The type of lesions produced on the cotyledons of the hazel nuts and on the tomato fruits strongly suggest their origin in insect punctures.

The fact that these yeasts parasitic on seed plants are so largely, if not exclusively, carried by insects, together with the fact that the other species of the group, *Monospora bicuspidata* and *Coccidiascus Legeri*, are themselves parasitic respectively on crustaceans and insects, presents, as noted above, an interesting situation bearing on the general problems of alternate hosts and insect carriers. The ordinary food substrata of yeasts are fruit juices capable of fermentation; and these juices are also a common food for insects, especially flies like *Drosophila*, the host of *Coccidiascus Legeri*. The situation seems naturally to suggest that yeasts now parasitic on green plants and carried by puncturing insects may have been derived from yeasts parasitic on insects, which in turn were derived from yeasts which live normally in fermenting fruit juices. Further data as to the possibility that the parasitic Nematosporas may multiply and perhaps pass the winter in *Nezara* is very much needed.

The asci of *Nematospora Phaseoli* may arise from individual yeast cells, from direct germination of the ascospores, from a conjugation of two ascospores, from single cells in the mycelial strands, and perhaps from the conjugation of two yeast cells. All of these cases are paralleled among the genera of the Saccharomycetes. The asci of the species of *Schizosaccharomyces* and *Zygosaccharomyces* most commonly arise from the conjugation of two bud cells, yet the individual cells often develop into asci without conjugating. In *Saccharomyces Ludwigii*, *Willia Saturnus* and yeast *Johannisberg II*, the asci usually develop

from single bud cells and the ascospores conjugate two by two at the time of germination. Budding from the zygospore thus formed usually follows, but it is not uncommon for asci to develop directly from a germ tube which arises from the conjugated pair. The single ascospores may also develop directly into asci. The asci of *Saccharomyces Cerevisiae* develop from single cells as a rule but, according to Hansen, they may also develop from the conjugation of two ascospores or the single ascospores may germinate directly into asci. In addition to the above methods of forming asci, *Saccharomyces Ludwigii*, *Pichia membranaefaciens*, and the yeast from pulque often form a mycelium on the surface of old cultures in which each cell of the hyphae may develop into an ascus with the normal number of ascospores.

Parallel cases are also to be found among the Endomycetaceae. In *Endomyces capsularis*, the asci are formed from single cells in the mycelium, but in some cases the ascospores form a bud or a germination tube that develops directly into an ascus. The asci of *Endomyces javanensis* develop from the individual yeast cells or from single cells in the mycelium. The asci of *Eremascus fertilis*, *Endomyces fibuliger*, and *Endomyces Magnusii* commonly develop from a conjugation of two cells in the mycelium, but they may also develop from single cells without conjugating.

It is obvious from all this diversity in the method of ascus formation in the various groups that, in so far as the process is associated with sexuality, modified and parthenogenetic conditions are suggested. The material is certainly very favorable for the study of the effects of environmental conditions on the occurrence and the significance in the life cycle of sexual cell fusions.

The data here presented are also of fundamental significance in connection with our conceptions of alternation of generations in both the lower fungi and the algae. The Nematosporas are relatively so favorable for cytological study that they constitute especially good material for the study of all these problems.

Summary

1. *Nematospora* is parasitic on a wide range of plants, but the Leguminosae predominate in the list.

2. Infection is associated with the punctures of particular insects.

3. The vegetative phase of *Nematospora* is typically yeast-like as a rule, but when grown in tap water, distilled water, or a very dilute solution of beer wort, a rudimentary mycelium is produced. A rudimentary mycelium is also produced around the borders of old colonies on solid media.

4. In the method of formation of its ascus *Nematospora* is in conformity with typical Ascomycetes in respect to the differentiation of spore-plasm and epiplasm, the triple division of the primary ascus nucleus, and the number of ascospores formed. The spores are formed by free cell formation.

5. The nuclear divisions in the ascus are typically mitotic. A spindle is formed and four or five chromosomes can be distinguished in the equatorial plate stage of the first and second divisions. The same number apparently holds for the third division, but the minuteness of the figures makes this a difficult matter to determine with certainty.

6. *Nematospora* is apparently rather closely related to the genera *Monospora* and *Coccidiascus*, which it resembles morphologically, so that these genera appear to form a natural group under Saccharomycetes.

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Explanation of plates 7–9

All figures were drawn with a camera lucida and a Leitz ocular no. 4; FIGS. 1, 3, 5, 10, 12—18b, 20–24 with a Leitz 1/16 in. oil imm. objective, N. Ap. 1.32; FIGS. 2, 4, 6, 7, 8, 9, 11, 19, 22 with a Leitz 1/12 in. oil imm. objective, N. Ap. 1.30; FIGS. 25–33 witn Leitz objective no. 6, N. Ap. 0.82. The magnification is given with each figure.

Plate 7

FIG. 1. Young ascus witn a single nucleus. × 2042.

FIG. 2. Primary nucleus of the ascus; equatorial-plate stage, five chromosomes and a nucleole. × 1600.

FIG. 3. Part of a binucleate ascus, showing the chromatin strands radiating from a point on the nuclear membrane and a nucleole lying in the nuclear space. × 2042.

FIG. 4. A young uninucleate ascus. × 1600.

FIG 5. Portion of a 4-nucleate ascus, showing two of the mitotic figures with five chromosomes × 2042

FIG. 6. Uninucleate ascus with tne nucleus in the spireme stage. × 1600.

FIG. 7. Ascus with the eight young ascospores. × 1737.

FIG. 8. Binucleate ascus with the nuclei in the metaphase stage; nine chromosomes can be distinguished. × 1600.

FIG. 9. Ascus with four mitotic figures; four or five chromosomes and a nucleole can be distinguished in each figure. × 1600.

FIG. 10. Ascus with two mitotic figures; five chromosomes and a nucleole can be distinguished in each of these figures. × 1874.

FIG. 11. Mature ascus, showing the eight mature ascospores; the basal

cell of each ascospore contains a single nucleus, while the apical cell contains only masses of disintegration products. × 1737.

Plate 8

FIG. 12. Ascospore with three nuclei in the swollen upper region of the basal cell; the original nucleus of this cell apparently divided, giving two, and then one of these underwent a second division, resulting in the two smaller ones. × 2042.

FIG. 13. Germinating ascospore. Budding is taking place from the upper region of the basal cell; the upper cell contains masses of disintegration products. × 2042.

FIG. 14. Ascus with the eight young ascospores, two of which have begun to elongate. × 1737.

FIGS. 15a, 15b, 16. Ascospores germinating by the budding process. × 2042.

FIG. 17. Ascospore showing the swelling of the upper region of the basal cell preparatory to germination. × 1894.

FIGS. 18a, 18b. Mature ascospores. × 1874.

FIG. 19. Uninucleate bud cells. × 1082.

FIG. 20. Basal cell of an ascospore germinating directly into an ascus. × 2042.

FIG. 21. Same as 20, with the nucleus in the process of division. × 2042.

FIG. 22. Ascus formed by direct germination of the basal cell of the ascospore. The primary nucleus is undergoing division, and five chromosomes and a nucleole can be distinguished on the spindle. Note the contents of the apical cell. × 1600.

FIG. 23. Binucleate ascus formed by direct germination of the ascospore. × 2042.

FIG. 24. Second nuclear division in an ascus formed by direct germination of the basal cell of an ascospore. × 2042.

Plate 9

FIGS. 25, 26. Asci formed on the tips of mycelial strands. × 500.

FIG. 27. Intercalary asci before ascospores are formed. × 312.

FIG. 28. Intercalary asci. × 500.

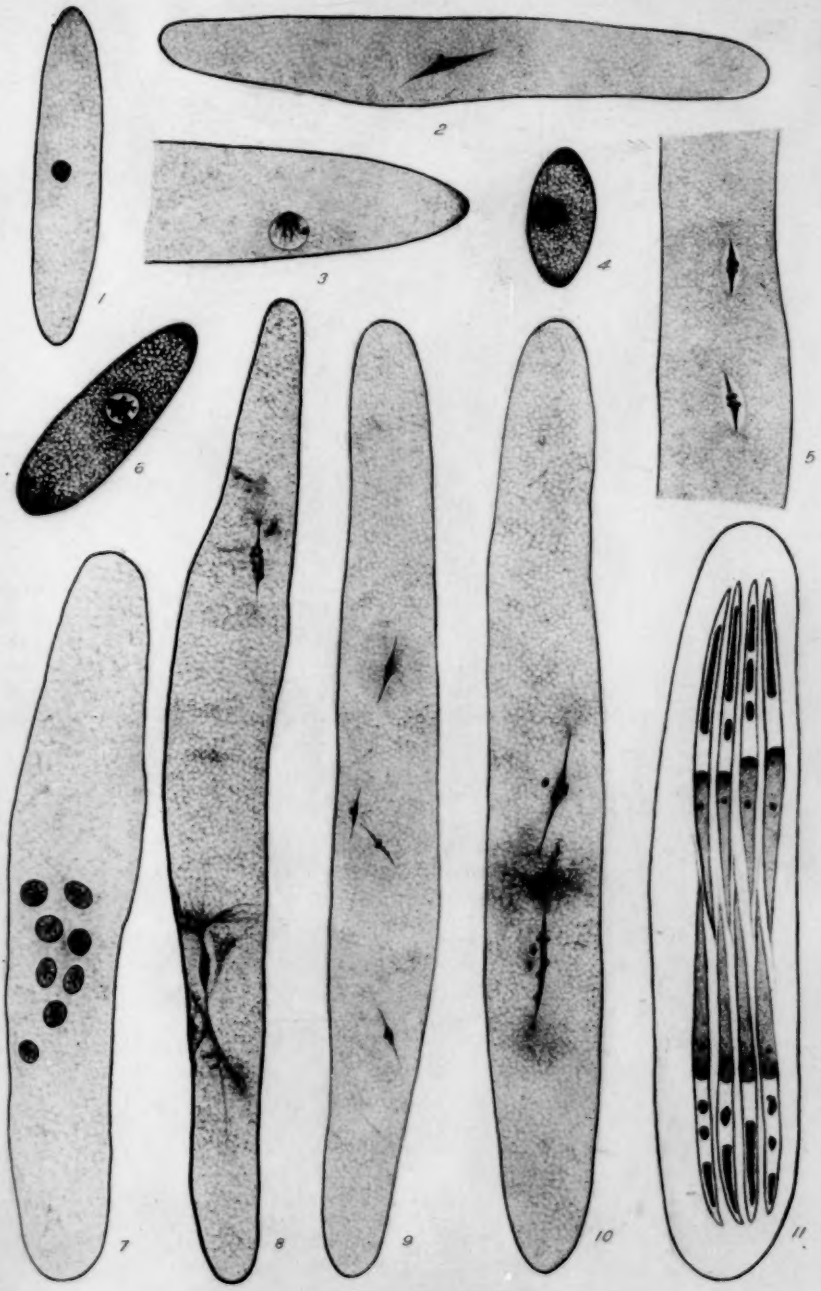
FIG. 29. Intercalary ascus with ascospores. × 312.

FIG. 30. Intercalary asci, one with ascospores. × 500.

FIG. 31. Germinating ascospore, showing the formation of intercalary asci. × 624.

FIG. 32. Two ascospores conjugating to form an ascus. × 500.

FIG. 33. An ascus formed by the conjugation of two ascospores. × 500.



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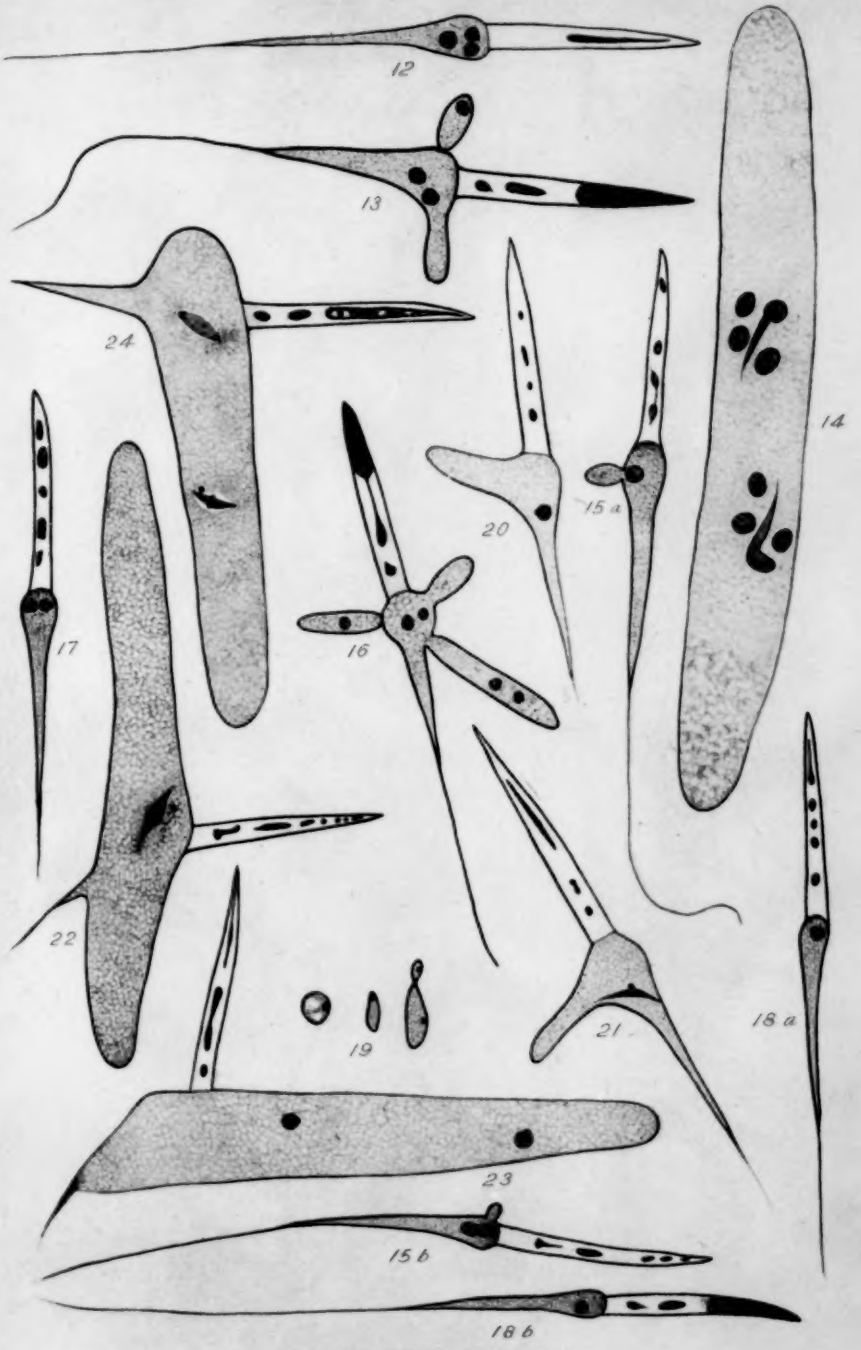

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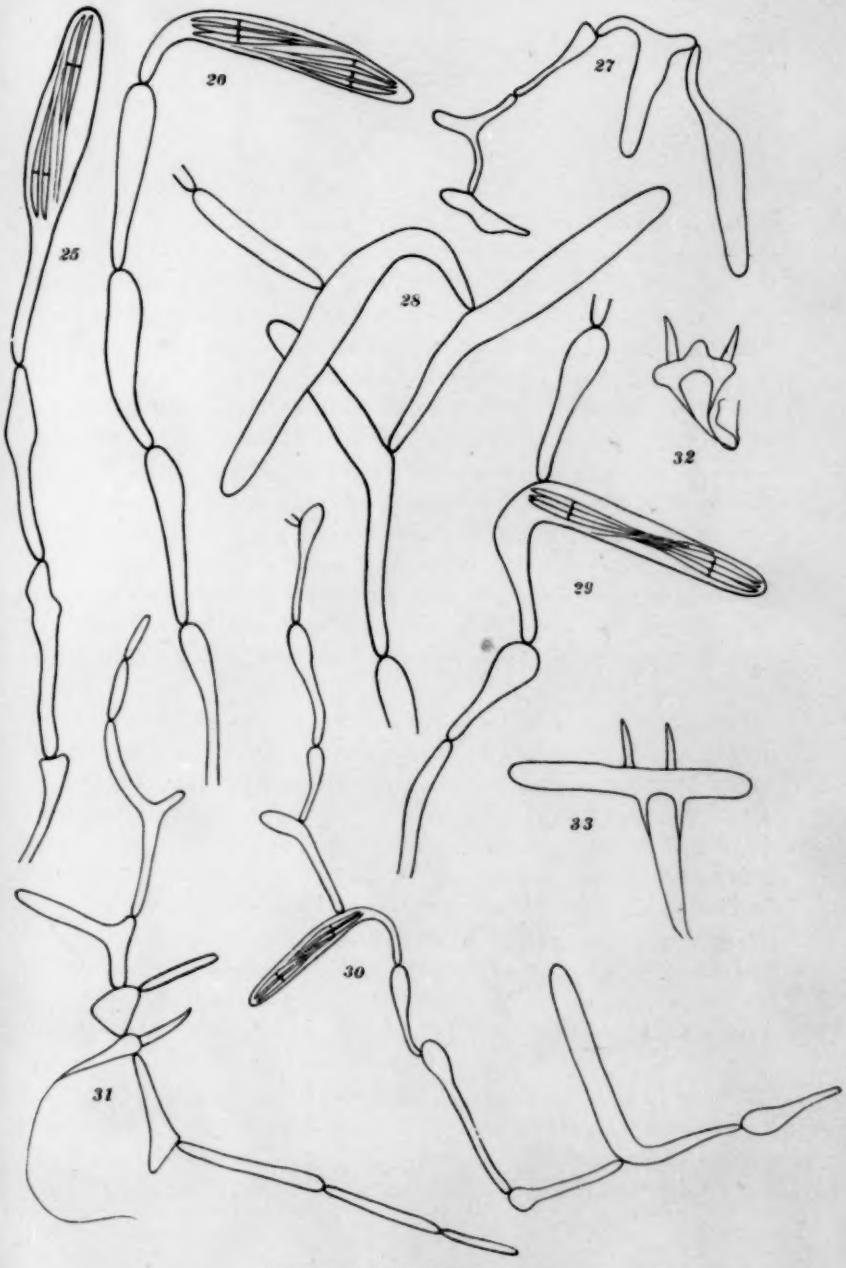

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Growth stimulation of Aspergillus niger by a vitamine B preparation*

NELLY JACOB SCHELLING

(WITH FOURTEEN TEXT-FIGURES)

The rôle of vitamines as accessory food substances in the nutrition of animals has become so important a question that it seems well to have more information upon their influence on the growth of plants. As will be seen from the brief survey of the literature which is given here our knowledge of the relation of vitamines and plants is rather meager. Of the published work, that of Lepeschkin (**18**) referred to later, most nearly coincides with the present account, though it by no means duplicates it. Lepeschkin's paper appeared, as will be seen by the date, at about the time that this study was completed.

It was the purpose of the present work to determine in how far the effects of a preparation of water soluble vitamine B from yeast upon the growth of *Aspergillus niger* are comparable to those obtained by stimulation with minute doses of chemical poisons. A few observations were also made on the effect of the same vitamine preparation upon wheat seedlings and upon *Lemna*.

Wildiers (**30**) in 1901 stated that he had found it impossible to obtain normal growth of *Saccharomyces Cerevisiae* in a synthetic inorganic medium if very small inoculations were made. Heavy inoculations resulted in abundant, normal growth of the organism. This at once gave Wildiers the clue to the unexplained discrepancies between the results of Liebig's and Pasteur's experiments on yeast, grown in mineral salt solutions. Biologists at the time took little notice of Wildiers' paper. The question did not become one of vital interest until Funk and other investigators discovered yeast to be a rich source of vitamine B. Most workers today hold that the Saccharomycetes require vitamines for their normal metabolism.

During the last few years a considerable amount of work has been done on the necessity of vitamines for the development of certain pathogens, especially *Bacillus Influenzae* (Pfeiffer's bacillus). According to Thjötta (**28**), Thjötta & Avery (**29**), and

* Contributions from the Department of Botany of Columbia University, no. 340.

Davis (**8, 9**) two growth accessory factors are involved in the growth of *B. Influenzae*. The two substances were found to differ in their thermostability (Davis **8, 9**). Both are reported to be contained in the blood corpuscles. Davis (**7**) tested the effects of vitamines from different sources on *B. Coli*, *B. typhosus*, *B. prodigiosus*, and *B. Diphtheriae*. In no case particular stimulation was observed.

What little work has been done on the vitamines in relation to the fungi leaves us pretty much in the dark as to their rôle and importance in these forms. Linossier (**19**) found *Oidium lactis* under certain conditions favorably influenced by the addition of vitamines to the nutrient solution. According to his observations, the fungus, if in very vigorous condition, can live perfectly normally on inorganic nutrients alone. Either it does not need vitamines for its growth, or it is capable of synthesizing them. If the vitality of the organism is in any way reduced (as by heat, age, etc.) the addition of growth promoting substances to the nutrient solution proved to have marked effects. Linossier furthermore observed that vitamines are absolutely essential to the growth of *Oidium lactis* if very small inoculations are made. Without vitamines in the solution, the fungus was found to germinate slowly and with difficulty. After about six days the controls caught up with the stimulated cultures.

Lepeschkin (**18**) confirmed Linossier's observation that the effect of vitamines upon fungi is evident during the first few days only. Working with *Aspergillus niger* and *Penicillium glaucum*, he noted very marked effects of the growth promoting substances during the early stages of germination, while there was practically no difference between the individual cultures after several days.

Lumière (**20**) denied any influence of the vitamines upon the growth of fungi. The "stimulation" observed after the addition of vitamines is attributed by this investigator not to the growth accessory substances as such, but to the addition of salts to poor synthetic media. The experiments which led Lumière to make such statements must be said to be inconclusive.

Goy (**16**) working with *Saccharomyces Cerevisiae*, *Mucor Mucedo*, *Aspergillus niger*, *Penicillium glaucum*, and *Bacillus Megatherium*, found these organisms to develop normally in mineral salt solutions. Much more proliferation was obtained

upon addition of small amounts of the medium in which previously had been grown either the same or a different species. The paper, unfortunately, leaves us in the dark as to the exact amount of old medium added, so that an interpretation of Goy's results is rather difficult. If minute quantities were used, the stimulation of proliferation suggests that from the organisms in question certain growth stimulating substances diffuse out into the medium. If, on the other hand, larger quantities of the old medium were added, the question of increased food supply might come into play.

Willaman (**31**) published a paper in 1919 on the function of vitamines in the metabolism of *Sclerotinia cinerea*, the brown rot fungus of plums and peaches. The organism did not develop unless vitamines from some source or other were added to the medium, a solution of mineral salts with the addition of asparagine and sucrose. Willaman found the amount of vegetative growth not in proportion to the juice added, large amounts of the latter causing slight inhibition of growth in the majority of cases. A large number of vitamine extracts which were examined, were found to stimulate the vegetative growth of *Sclerotinia*, but only very few induced reproduction. Furthermore, Willaman found the amount of sporulation not necessarily proportional to the amount of vegetative growth. These observations suggested the possibility of two different vitamines at work, one stimulating vegetative growth, the other one important in reproduction. Willaman, however, believes that a single vitamine is responsible for the stimulation of both vegetative growth and reproduction of *Sclerotinia cinerea*.

Bottomley (**1–4**) developed the theory that not only lower forms, but also green plants require growth promoting substances or auximones. He found that *Lemna minor* and several other aquatics did not develop normally in mineral salt solutions without the addition of auximones. These organic growth promoting substances were obtained from soil, as a result of the bacterial decomposition of peat, from cultures of *Azotobacter Chroococcum*, and *Bacillus radicicola*, as well as from manure (Mockeridge **21**). The identity of the auximones with vitamines is indicated by the fact that the phosphotungstic acid, and the silver fraction precipitate both substances from solutions. Auximones were found to have a marked stimulating effect

upon the rate of reproduction of *Lemna minor*. Cytological examination of stimulated plantlets showed larger nuclei, denser cytoplasm, and fewer, smaller air-spaces than the controls. Bottomley obtained analogous effects of auximones on wheat but these plants did not give as satisfactory results as *Lemna minor*. This is due to the fact that wheat, by virtue of having an endosperm, is not as dependent upon the medium. Furthermore, it has been proved conclusively that the dry wheat grain contains a small quantity of vitamines, while the germinating grains are very rich in these substances.

Rosenheim (**25**) tested the effects of auximones on the growth of *Primula malacoides*. After a treatment of three weeks the plants were found to have larger and darker leaves, as well as a greater abundance of flowers than the controls.

EXPERIMENTAL PART

The methods used were those that have been employed by many previous investigators and are consequently described but briefly here. The source of the vitamine used was the preparation known as Vitamine B Harris. Pyrex glassware was used in all cases for the culture dishes, as giving a glass with minimum solubility, and the water employed was distilled twice over a block tin coil. Conductivity tests showed that this water was of high degree of purity. The chemicals employed were those of Merck and Co., Reagent grade, and as a sugar source Pfanstiehl's sucrose was used.

Except in one case, mentioned later, the culture flasks used were of 150 cc. capacity and of the Erlenmeyer form. Fifty cubic centimeters of culture solution were used to each flask. This solution was made up by the formula of Pfeffer which has been largely employed for similar work. Its composition is as follows:—

KH_2PO_4	0.5 gram
$MgSO_4$	0.25 "
NH_4NO_3	1.0 "
Fe_2SO_4	trace
Sucrose	5.0 "
Water	100 cc.

The yeast vitamine solution was added from a standard solution made up fresh for each set of cultures. Two hundred milligrams

of the vitamine powder were weighed out and dissolved in four cc. of water. By means of a pippette delivering a drop of as nearly as possible one twentieth of a cubic centimeter volume, certain definite quantities were added as follows:—

	Vit. sol. added	*Equivalent of*	%	*H_2O added*
Culture A	(Control) 0			16 drops
" B	1 drop	2.5 mg. dry vit.	.05	15 "
" C	2 drops	5.0 " " "	.10	14 "
" D	4 "	10.0 " " "	.20	12 "
" E	10 "	25.0 " " "	.50	6 "
" F	16 "	40.0 " " "	.80	0 "

The water was added to keep the surface areas and concentrations of nutrients equal in all cases. The cultures were then inoculated with the *Aspergillus niger* spores, and placed in the incubator, the temperature of which was kept at 35° C. After three and a half days the mycelium felts were filtered off on weighed filter paper, washed with distilled water, and dried for forty-eight hours in a hot air drying oven at about 70° C.

Vitamine B was found to be a stimulant to both vegetative growth and reproduction of *Aspergillus niger*. TABLE 1 shows the stimulation of growth, measured by the increase in the dry weight of the mycelial felts.

TABLE 1. DRY WEIGHTS OF STIMULATED ASPERGILLUS CULTURES

Control	*2.5 mg.*	*5 mg.*	*10 mg.*	*25 mg.*	*40 mg.*
.482 gr.	.491 gr.	.475 gr.	.603 gr.	.621 gr.	.713 gr.
.289	.394	.399	.490	.452	.631
.424	.487	.492	.405	.463	.554
.408	.471	.344	.436	.445	.406
.344	.410	.425	.508	.491	.452
.394	.371	.408	.584	.584	.608
.345	.417	.386	.526	.676	.689
.380	.320	.399	.398	.442	.488
.398	.396	.402	.510	.483	.593
.366	.415	.367	.426	.512	.582
.357	.441	.388	.567	.567	.579
.355	.387	.399	.437	.538	.519
.419	.452	.438	.454	.572	.565
.393	.404	.437	.442	.503	.625
Average dry weight .382 mg.	.418 mg.	.411 mg.	.484 mg.	.524 mg.	.571 mg.
Average increase over Control	9.4%	7.5%	26.7%	37.1%	49.4%

The results expressed in this table show at once some very striking differences as compared with those obtained by stimulation with minute quantities of toxic substances, such as zinc sulphate. In the case of the vitamine cultures the increase in dry weight is very gradual. In a typical zinc stimulated culture, on the other hand, the smallest dose of the salt used produces a very marked increase in weight which is comparatively little less than the maximum yield.

High concentrations of vitamines affect the organisms in one of three ways, as reported in the literature:

(1) For certain organisms, for instance the yeasts, there is an optimum concentration of the vitamine, beyond which point the curve drops (Euler & Myrbäck **10**, Funk & Freedman **15**).

(2) Only a definite amount of vitamine is available to the organism; higher concentrations have no effect. Thus, Lepeschkin (**18**) states that yeast requires only a very small amount of vitamines, which quantity it was found needless to increase.

(3) Chick & Hume (**5**) apparently do not believe in a maximum concentration of vitamines. They found birds to be in a "remarkably fine condition," if fed on polished rice, and an excess of vitamines.

Graphic representation of the results of the stimulation of growth of *Aspergillus niger* by vitamine B shows a smoothly ascending line, thus suggesting a reaction of type 3 (see FIG. 1).

A reaction of type 1 could not be induced, even if exceedingly high concentrations of vitamines were used. A series of experiments was carried out to establish this point. Vitamine powder was added to the culture medium minus the ammonium nitrate. The results are summarized in TABLE 2.

TABLE 2. DRY WEIGHTS OF THE CULTURES MINUS NH_4NO_3

	50 mg.	*100 mg.*	*125 mg.*	*200 mg.*	*250 mg.*	*500 mg.*
		.477 gr.		.604 gr.	.685 gr.	.900 gr.
	.300 gr.	.458		.545	.618	
					.635	.950
					.690	.958
			.554 gr.		.791	.898
			.500		.728	.996
			.596		.846	1.276
			.585		.699	1.097
Average	.300 gr.	.467 gr.	.558 gr.	.574 gr.	.711 gr.	1.010 gr.

Conidia formation in the case of the 125 mg. vitamine powder culture occured much earlier than in the case of the control. As the vitamine supply was doubled and quadrupled, sporulation was retarded. Even when the supply of vitamine powder was as high as 500 mg. conidia were formed at an earlier stage than in the controls without vitamine.

The above experiments not only prove that high concentrations of vitamines do not inhibit growth and reproduction of *Aspergillus niger*, but they also show that vitamine B is an excellent nitrogen source for the fungus.

Several sets of experiments were carried out in order to determine whether the degree of stimulation would show any variation if the cultures were grown for different lengths of time.

TABLE 3. PERIOD OF GROWTH: FORTY HOURS*

	Control	*10 mg. Vit./50 cc.*	*40 mg. Vit./50 cc.*
	.455 gr.	.515 gr.	.603 gr.
	.448 "	.505 "	.577 "
Average	.451 gr.	.510 gr.	.590 gr.

TABLE 4. PERIOD OF GROWTH: TWELVE DAYS

Control	*2.5 mg. Vit./50 cc.*	*5 mg.*	*10 mg.*	*40 mg.*
.532 gr.	.611 gr.	.542 gr.	.632 gr.	.698 gr.

Graphic representation of the results expressed in TABLES 1, 3 and 4 shows three gradually rising lines which run more or less parallel to one another (FIG. 1).

The results expressed in TABLE 4 are not in accord with those obtained by Lepeschkin (18), and Linossier (19). The former states that the effects of vitamines on *Penicillium glaucum* and on yeast are noticeable only during the first few days, when the organism has an excess of food, while after several days no difference between the individual cultures was noted. Linossier found the same thing to hold true for *Oidium lactis*.

Considerable differences were noted between the microscopic appearance of vitamine and zinc cultures. The mycelium of the latter is leathery, knotted, and thrown into heavy folds. The mycelial felts in the case of the vitamine cultures are practically

* In order to have as large a surface area as possible, 250 cc. Erlenmeyer flasks were used for this particular experiment.

smooth, very thick and tenuous. Vitamine B accelerates the formation of conidia, while stimulants in the form of toxic substances retard or suppress sporulation. The addition of small quantities of tomato juice to the medium was found to have exactly the same effect on the time of fructification as the addition of yeast vitamine. It appears that in the case of *Aspergillus niger* early conidia formation is one of the main reactions caused by vitamines.

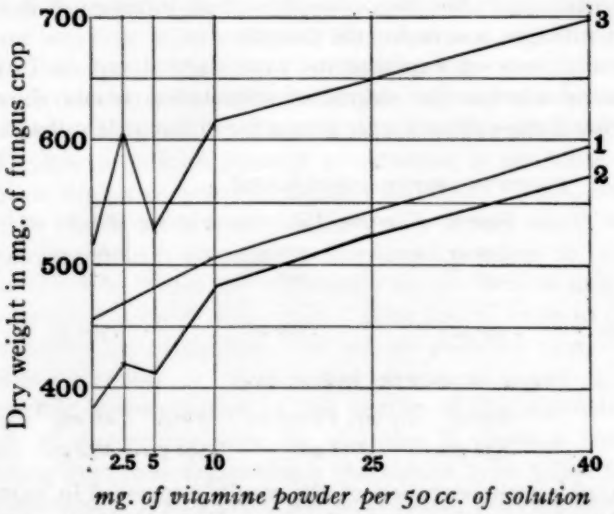


FIG. 1. 1, growth in forty hours; 2, growth in three and one-half days; 3, growth in twelve days.

To study the stimulation of the fungus during its early stages of growth, the percentage of germinating spores within a given time had to be determined. Hanging drop cultures in the usual Van Tieghem cells were used for this purpose and the culture solutions prepared as for the flasks. As few spores as possible were inoculated into the hanging drop. The cultures were grown in an incubator at 35° C. The results expressed in TABLES 5 and 6 suggest that the maximum reaction of vitamines on *Aspergillus niger* occurs within the first five hours after inoculation.

In another series of experiments vitamine powder was added to double distilled water, instead of to the culture solution. Analogous results to those expressed in TABLES 5 and 6 were obtained, and are set forth in TABLES 7–9.

TABLE 5. PERCENTAGE OF GERMINATING SPORES, FIVE HOURS AFTER INOCULATION IN NUTRIENT SOLUTION + VITAMINE:

	Control	*2.5 mg.*	*5 mg.*	*10 mg.*	*25 mg.*	*40 mg.*
	52.1	72.4	72.0	79.0	82.7	81.3
	56.3	66.6	71.1	78.2	84.3	84.1
	65.3	70.7	73.8	77.8	90.1	93.2
	64.4	76.4	84.6	86.0	91.2	91.4
	44.2	83.6	81.2	82.7	89.0	90.2
	52.9	75.6	81.5	82.3	81.3	88.1
Average	55.8	74.2	77.3	81.0	86.4	88.05

TABLE 6. PERCENTAGE OF GERMINATING SPORES, NINE HOURS AFTER INOCULATION IN NUTRIENT SOLUTION + VITAMINE:

	Control	*2.5 mg.*	*5 mg.*	*10 mg.*	*25 mg.*	*40 mg.*
	95.9	97.8	96.1	96.0	97.9	98.9
	82.3	92.8	94.2	92.9	97.9	97.0
	83.1	92.7	92.2	94.0	96.8	97.0
Average	87.1	94.4	94.19	94.3	97.5	97.3

TABLE 7. PERCENTAGE OF GERMINATING SPORES, FIVE HOURS AFTER INOCULATION IN DISTILLED WATER + VITAMINE:

	Control	*2.5 mg.*	*10 mg.*	*25 mg.*	*40 mg.*
	9.1	34.8	50.6	59.9	65.7
	7.2	25.5	45.3	50.3	67.0
	10.1	25.2	44.0	53.3	62.1
Average	8.8	28.5	46.6	54.5	64.9

TABLE 8. PERCENTAGE OF GERMINATING SPORES, TEN HOURS AFTER INOCULATION IN DISTILLED WATER + VITAMINE:

Control	*2.5 mg.*	*10 mg.*	*25 mg.*	*40 mg.*
17.6	44.0	55.4	61.8	83.3

TABLE 9. PERCENTAGE OF GERMINATING SPORES, TWENTY-FOUR HOURS AFTER INOCULATION IN DISTILLED WATER + VITAMINE:

	Control	*2.5 mg.*	*10 mg.*	*25 mg.*	*40 mg.*
	28.1	68.6	72.2	80.3	93.5
	25.9	67.7	73.4	81.9	88.7
Average	27.0	68.1	72.8	81.1	91.1

The results set forth in TABLES 5–9 are summarized graphically in FIG. 2.

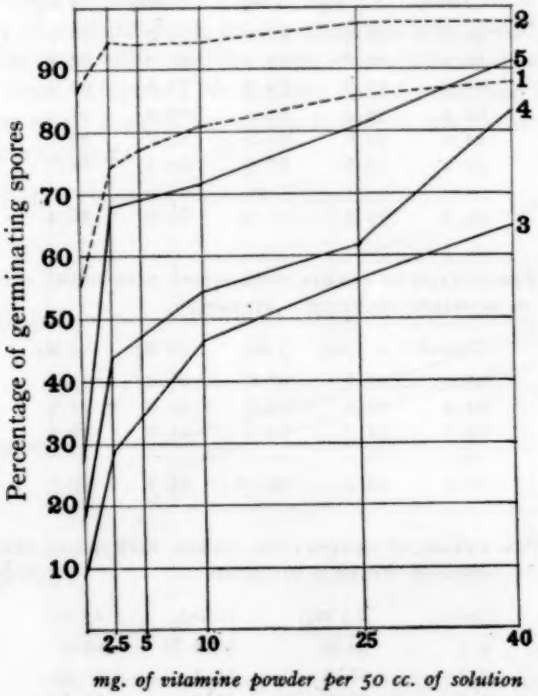


FIG. 2. 1 and 2 in nutrient solution + vitamine, after five and nine hours respectively. 3, 4 and 5 in distilled water + vitamine, after five, ten, and 24 hours respectively.

The above results cannot necessarily be interpreted to mean that vitamines, and not the culture medium, are the all important factors in the germination of *Aspergillus*. Evidently, the spores of this fungus contain a very limited amount of stored food, which enables them to germinate in double distilled water. The vitamines, in this case, besides stimulating germination, certainly act as a food supply. The relative importance of these factors will be discussed later.

Microscopic examination of young germinating spores grown in nutrient solution with and without vitamines revealed certain marked morphological differences. The spores of the vitamine cultures were found to develop long, straight hyphae which

branched little. The branchlets are short, blunt, and uneven in diameter. In the controls the hyphae are comparatively short, but they show profuse branching. The difference between the different cultures does not become evident until at least nine hours after inoculation. FIGS. 3–7 illustrate this point.

Very marked differences were observed in a thirty-six hour culture (FIGS. 8–13). The control exhibited normal primary and secondary branching. Spores grown in the highest concentration of vitamines (40 mg./50 cc. of solution, circa 0.8 per cent) showed long straight hyphae, at the ends of which developed the usual primary and secondary branchlets, short and peculiarly blunt. Long, slender hyphae with exceedingly long cells grew out from these branches. Anastomosing between these secondary hyphae was frequently observed. The whole gave an impression as though growth, for some reason, had been temporarily arrested, and then suddenly resumed. Between the two extremes—control and most highly stimulated culture—all intermediate stages were found (FIGS. 6–11).

Anastomosing between different hyphae was occasionally observed in the stimulated cultures, but never in the controls It was very abundant in the cases where the fungus was given vitamine powder as its only nitrogen source, and thus appears to be one of the characteristic effects of vitamines.

The determination of the economic coefficient of sugar showed that, in this regard, vitamine cultures behave in the same way as cultures which are stimulated by small doses of toxic salts: stimulation results in the raising of the economic coefficient of sugar, though in small amount.

TABLE 10. MG. MYCELIUM FORMED PER 100 MG. OF SUGAR USED

	Control	*5 mg.*	*10 mg.*	*25 mg.*	*40 mg.*
	35.4 mg.	35.88 mg.	35.83 mg.	36.2 mg.	38.1 mg.
	37.1	41.7	49.5	42.6	45.2
	36.3	34.1	37.0	41.4	39.3
	35.2	38.7	38.0	41.5	42.6
Average	36.0 mg.	37.5 mg.	40.0 mg.	40.4 mg.	41.3 mg.

Richards (**23**) explains the raising of the economic coefficient of sugar as follows: ". . . the direct action of irritant substances . . . which produce an increased growth of certain fungi is to

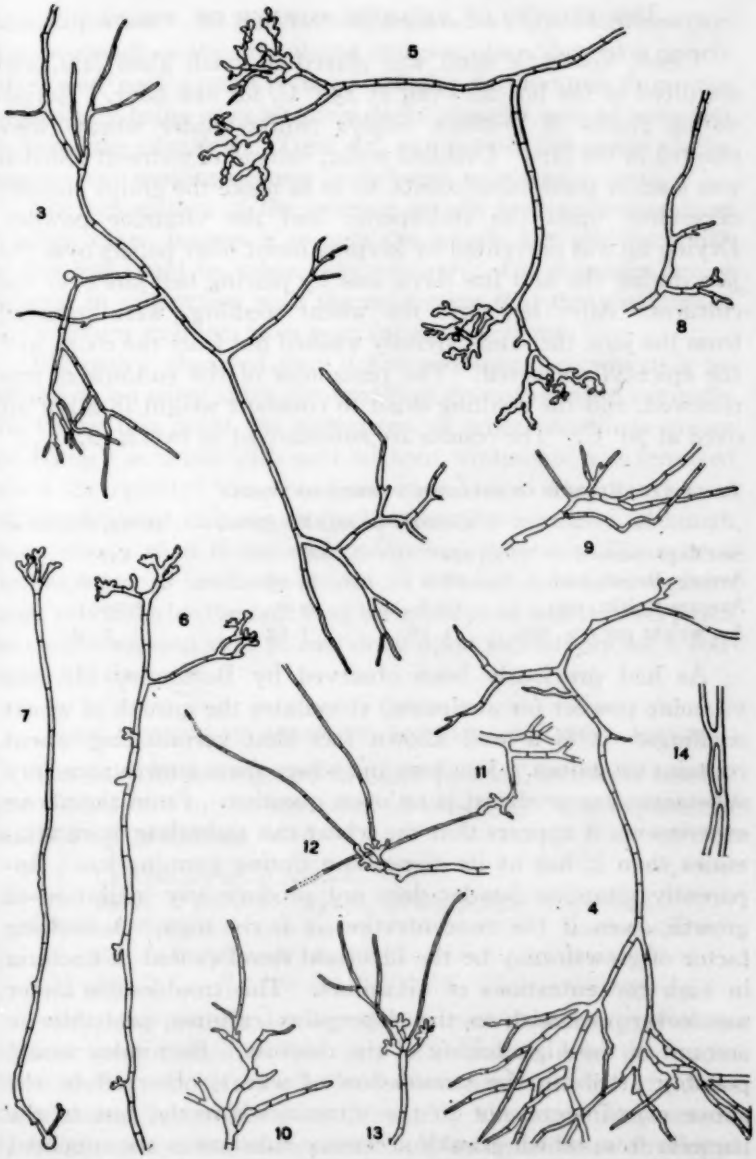
enable the latter to dispose more economically of the sugar used (i.e. to raise the economic coefficient of sugar) thereby permitting a more rapid production of dry substance in a given time." In the case of the vitamine cultures another explanation might possibly be resorted to: Funk (**13**) suggested that vitamines play an important rôle in the assimilation of carbohydrates. From his point of view it might be argued that in the vitamine cultures the fungus is able to assimilate the sugar more easily, and to use it more economically.

Richards (**24**), discussing the conidia formation in relation to the economic coefficient of sugar states that "Whether it is the more economical working of the protoplasm which inhibits the formation of conidia, or whether the absence of the latter results in less waste of energy in metabolism is perhaps a question, though probably most would agree that the spore-forming process is one that demands a greater expenditure of energy than the mere vegetative growth of the hyphae." This explanation holds good in all cases where stimulation expresses itself in increased vegetative growth, and inhibition of the reproductive factor. For vitamine cultures, where both phases of growth are stimulated, the explanation of the phenomenon has evidently to be sought elsewhere.

The acidity of the stimulated cultures was found to be less than that of the controls. It was determined by titration with standardized N/20 sodium hydroxide. No determinations of the hydrogen-ion concentrations were made. The filtrate left at the end of the experiment was made up to 100 cc., and an aliquot portion titrated, phenolphthalein being used as indicator. Upon deducting the original acidity of the medium from the final acidity, the acid formed in the metabolism was determined. The value of this amount has to be considered as relative only, inasmuch as some acid may be broken down further, or taken up again in the anabolism of the fungus.

TABLE 11. CC. N/20 ACID FORMED PER 100 MG. OF MYCELIUM (DRY WEIGHT)

	Control	*2.5 mg.*	*5 mg.*	*10 mg.*	*25 mg.*	*40 mg.*
	3.24	2.33	2.95	1.70	1.60	1.02
	1.64	1.81	1.41	1.13	1.38	1.35
	2.34	2.40	2.15	2.37	1.97	1.54
Average	2.40	2.18	2.17	1.73	1.65	1.30



FIGS. 3–14. 3. Control, nine hours after sowing. 4–7. Same, but with 2.5-, 5-, 10-, 25-, and 40-mg. vitamine preparation per 50 cc. culture solution respectively. 8. Control, growing tips of hyphae thirty-six hours after sowing. 9–13. Same, but with 2.5-, 5-, 10-, 25-, and 40-mg. vitamine per cc. culture solution respectively. 14. Anastomosing hyphae as seen in cultures of high vitamine content.

THE EFFECTS OF VITAMINE POWDER ON WHEAT

Clean, synthetic sand was placed in small glass jars, and sterilized in the hot air oven at 150° C. for one hour. Germinating grains of *Triticum vulgare* (white winter wheat) were planted in the jars. Distilled water, instead of nutrient solution was used in these experiments, so as to make the grains entirely dependent upon the endosperm, and the vitamine powder. Drying up was prevented by keeping moist filter papers over the jars during the first few days, and by placing bell-jars over the cultures. After ten days the wheat seedlings were removed from the jars, the sand carefully washed out from the roots, and the epicotyl measured. The remainder of the endosperm was removed, and the seedling dried to constant weight in a hot air oven at 70° C. The results are summarized in TABLE 12.

TABLE 12. EFFECTS OF VITAMINE POWDER ON WHEAT

	Control	*20 mg. Vit./50 cc.*	*40 mg. Vit./50 cc*
Seedlings counted	172	228	137
Average length, cm.	11.1	13.06	13.8
Average weight, mg.	19.8	22.0	20.3
Av. weight per cm., mg.	1.78	1.67	1.36

As had previously been observed by Bottomley (**2**), the vitamine powder (or auximone) stimulates the growth of wheat seedlings. It is a well known fact that germinating wheat contains vitamines. Just how and where these growth accessory substances are produced is an open question. From the above experiments it appears that the wheat can assimilate more vitamines than it has at its disposition during germination. Apparently vitamine powder does not produce any inhibition of growth, even if the concentration is fairly high. A limiting factor of growth may be the luxuriant development of bacteria in high concentrations of vitamines. This troublesome factor was never met with in the *Aspergillus* cultures, probably on account of the high acidity of the medium. Bottomley would possibly attribute the stimulation of wheat, observed in the above experiments, not to the vitamines directly, but to the bacteria from which growth accessory substances are supposed to diffuse out into the medium.

The macroscopic appearance of the epicotyls of seedlings grown in distilled water with or without vitamines showed very

little difference. All the plants appeared dark green and healthy. The seedlings of the stimulated cultures often showed a poorly developed root system. They differed furthermore from the controls by being very weak: a slight draught proved sufficient to bend the plantlets. Time did not permit the study of the anatomy of seedlings grown in different solutions.

The calculation of the average weight per centimeter is of relative value, inasmuch as only the length, but not the width of the leaf could be taken into account. The numbers are of interest in connection with the suggestion that the seedlings of the vitamine cultures have poor supporting tissue.

Bottomley observed that if the endosperm of wheat is removed at an early stage the seedlings do not develop normally To verify this point the endosperm of wheat seedlings grown on Knop's solution with and without vitamines was removed when the epicotyl was about one inch long. The plantlets of all the different cultures seemed to develop normally, although more slowly than if the endosperm was present. The normal development of seedlings grown in mineral solutions explains itself probably by the fact that the embryo of wheat is very rich in vitamines, and that it can draw upon this supply for a considerable time.

The effects of vitamine powder on Lemna minor

The cultures were grown in black painted, sterilized battery jars. Knop's solution was used as medium. The experiment was set up as follows:

I. Control: 600 cc. of solution.
II. 600 cc. of solution + 240 mg. of Vit. powder, unsterilized.
III. 600 " " " + 240 " " " " sterilized.
IV. 600 " " " + 480 " " " " unsterilized.
V. 600 " " " + 480 " " " " sterilized.

Twenty-five mature *Lemna* plants, each consisting of three leaflets, were placed in each jar. The culture was grown for three weeks. Once a week the leaflets were counted, the solutions changed, and the jars thoroughly cleaned. This proved to be necessary, inasmuch as a thick bacterial scum developed regularly in all the solutions containing any vitamine. Sterilization of the media helped little: the bacteria were apparently introduced with the *Lemna* plants, and the acidity of the culture

solution was not sufficiently high to check the development of these organisms.

After the first week the control plants had practically all broken up into single leaflets which looked yellowish and sickly. The plants grown in solutions II, III, IV and V, on the other hand, were dark green. Each one consisted of three to five leaflets. As TABLE 13 shows, the number of control plants increased hardly at all during the two following weeks, while the reproduction of the plants of the remaining cultures appeared markedly stimulated.

TABLE 13. STIMULATION OF LEMNA MINOR

	Control	*II*	*III*	*IV*	*V*
One week	96	119	128	146	141
Two weeks	110	162	164	169	199
Three weeks after inoculation	126	189	197	243	297

Contrary to expectation, the vitamine culture during the first week of growth showed marked stimulation of vegetative reproduction, while the second week brought a slowing down of this process. The maximum reproduction in the case of IV and V was obtained during the third week.

These observations only partly confirm the results of Bottomley, who found his maximum reaction to occur during the second and third weeks. Bottomley's tables furthermore show a much more marked stimulation than was obtained in the above experiments. The luxuriant development of bacteria in the vitamine cultures constitutes possibly a limiting factor on the rate of growth. On the other hand, it might be argued that since Bottomley obtained his growth promoting substances from bacterial cultures, the stimulation in the above experiments might be due, not to the vitamines as such, but rather to substances which diffuse into the medium from bacterial cells. Reasoning from analogy with the *Aspergillus* and wheat cultures, this suggestion will hardly hold good.

DISCUSSION

Aspergillus niger develops normally in Pfeffer's solution without any accessory substances. Both vegetative growth and reproduction are accelerated by the addition of small amounts of vitamine B to the medium. The main stimulating effects of

the growth accessory substances upon *Aspergillus niger* were found to occur during the first five hours after inoculation. The ratio of the dry weights of the mycelial felts of the stimulated cultures, and the controls is practically constant, whether the cultures be reaped forty hours, three and one-half days, or twelve days after inoculation. In this regard, the results of the experiments described in this paper are at variance with the observations of Lepeschkin and Linossier, who found that vitamines have a stimulating effect upon *Penicillium glaucum* and *Oidium lactis* only during the very earliest stages of growth.

In the opinion of Steinberg (**27**) the cause of stimulation is the increased acidity of the medium, due to the addition of certain substances. The results of the experiments described in this paper might be interpreted along this line (TABLE 11), but there are reasons to believe that the matter is more complicated.

The effect of vitamine B upon *Aspergillus niger* may be catalytic. If Funk's conception that vitamines are of utmost importance in the carbohydrate metabolism is correct, the sugar in the stimulated cultures would be more available to the organism. The economic coefficient of sugar would thus be raised: the different phases of growth could be carried on with greater ease and rapidity. To prove the point whether vitamine B acts as a catalyst in the sense that it renders the carbohydrate more available to the organism, it would be necessary to determine the amount of vitamine still present at the end of an experiment. For obvious reasons, such a determination is involved with great difficulties, if not impracticable.

It is most probable that the effects of vitamine B are those of an increased organic food supply, or that the substance acts as a stimulant in the sense that small amounts of toxic substances act as stimulants.

Several considerations are in favor of the former conception. Stimulation of the vegetative growth, expressed by the increased dry weights of the mycelial felts is much more nearly quantitative than is the case for instance with a typical zinc culture (TABLES 1, 3, 4, FIG. 1). Furthermore, *Aspergillus niger* grows profusely in media containing vitamine powder as the only nitrogen source (TABLE 2). There is no definite optimum nor maximum concentration of the vitamine in the culture solution. Fleming (**11**) suggested that the addition of organic nitrogen to

a medium containing only inorganic nitrogen might cause stimulation. To determine the correctness of this conception it will be necessary to make quantitative analyses of the nitrogen content, as described by Latham (17). On the other hand stimulation is found with very small doses of the vitamine preparation, where the added nitrogen content can hardly play a significant rôle.

However, taking into consideration the raising of the economic coefficient of sugar, and the lowering of the total acidity, it seems more logical to suppose that the effects of vitamine B are similar to those obtained by the addition of minute doses of toxic substances to the culture medium. The main difference between vitamine and zinc cultures lies in the fact that, in the case of the former, sporulation is never suppressed, nor even inhibited, but on the contrary stimulated, while, at the same time, the development of the mycelium is not as markedly stimulated. These two observations do not constitute a point of vital difference between the vitamine and the zinc cultures. They may easily be explained by supposing that two factors are brought into play by the addition of vitamine powder to the medium, one factor stimulating vegetative growth, the other one important in reproduction. It is easily conceivable that the vegetative factor is limited by the reproductive factor. It appears that the vegetative factor is at its optimum if the concentration of the vitamines is lowest, i.e. at 0.05 per cent. TABLE I and FIG. 1 show that comparatively higher weights of the mycelia are obtained with this concentration than with the next higher. A further argument in favor of the double stimulation effect is the fact that the germination of spores is markedly stimulated by very low concentrations of vitamines (TABLES 5, 6). During these earliest stages of growth the vegetative factor alone is at work.

These arguments lead the writer to believe that the effects of water soluble vitamine B upon the vegetative growth of *Aspergillus niger* are similar to those produced by small doses of toxic substances.

This work was done under the guidance of Professor Herbert M. Richards, for whose kind advice and criticism the writer is greatly indebted.

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The embryogeny of Cephalotaxus Fortunei*

JOHN T. BUCHHOLZ

(WITH PLATE 10 AND TWO TEXT FIGURES)

The embryogeny of *Cephalotaxus* is of very great interest in a study of the comparative morphology of conifers. *Cephalotaxus* is usually included among the Taxineae, but the embryo has been reported as having certain points of comparison to the Abietineous type of embryo: it has a terminal cap which may be homologous with the embryo cap of Araucarians and it furnishes a key to the homologies between the embryos of the other Taxineae and Podocarpineae. The conclusions reached in this paper apply to embryogeny only, and it is fully realized that they must be considered in connection with other morphological facts in any final decision concerning the proper affinities of this genus. As such we believe that comparisons based on the embryogeny should be among the most important considerations, and this study aims to emphasize this phase in the morphology. The interpretation given here, while a new one, was suggested in its main outline in the writer's resumé of conifer embryogeny (1920) and finds much additional support from the first-hand examination of this material.

MATERIAL AND METHODS

The material for this investigation was kindly furnished by Dr. W. C. Coker in 1921, from trees growing in the Arboretum at Chapel Hill, North Carolina. Living twigs of *Cephalotaxus Fortunei* were sent to the writer in four collections, from June 19 to August 3, 1921. The gametophytes were removed and their embryos dissected out in the living condition with the aid of a Greenough binocular microscope under a sugar solution of about ½ gram molecular concentration. These were placed immediately in a killing and preserving fluid consisting of 6 per cent formalin in 50 per cent alcohol. In this condition they were preserved for several years. In the technique of mounting,

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the embryos were washed and then stained for fifteen minutes in Delafield's haemotoxylin, de-stained slightly with acidulated water, after which they were again washed, placed in 10 per cent glycerine and left for several days in the open, protected from dust, until the glycerine became fully concentrated. The glycerine was washed out with several changes of 95 per cent alcohol, from which they were transferred to absolute alcohol. From this they were mounted in diaphane (a new mounting medium manufactured by the Will Corporation, Rochester). A previous test of this mounting medium with algae indicated that it would be best to transfer the material into diaphane gradually, first placing it in a mixture of half diaphane, half absolute alcohol. The embryos were therefore placed on a slide from the absolute alcohol and this 50:50 solution dropped on them immediately. The alcohol evaporates rapidly, and may be followed in from one to three minutes by the pure diaphane medium, and covered.

This medium seems to possess most of the advantages of Venetian turpentine used in the writer's previous work, and saves a little time. It also hardens faster than Venetian turpentine, and does not emulsify when moisture is absorbed from the air. The blue stain of the haemotoxylin is rendered brown, which is of no disadvantage for direct observation and has some distinct advantages in photographic work. Sections were made of the embryos in the last collection. These were killed in formalin-alcohol, cut in paraffin, stained with Haidenhain's haemotoxylin, counterstained with gold orange and mounted in Canada balsam.

The photomicrographs were made with an Edinger apparatus, using Bausch and Lomb Micro-tessars stopped down, and the low power microscope combination of the apparatus (⅔ inch objective and 8× aplanoscopic ocular) with Cramer's contrast plates.

Previous investigations

Cephalotaxus has been investigated morphologically by Strasburger (1879), Arnoldi (1900), Coker (1907), and Lawson (1907). The various other investigations of this species did not include accounts of the embryogeny. Strasburger's observations begin with the organized proembryo, while Arnoldi, Coker and Lawson included earlier stages. The first three of these in-

vestigators included only *Cephalotaxus Fortunei*, while Lawson was largely concerned with *Cephalotaxus drupacea*, though he also examined material of the former. There seem to be no important differences between these two species. The proembryo stages are briefly outlined below in order to make the present description more complete, but the writer's observations were confined to the stages after the walls were formed and the suspensor cells were beginning to elongate.

Proembryo

The proembryo of *Cephalotaxus* has been described by the above mentioned investigators and in the main outline these accounts are in substantial agreement. A good summarized account of this is given by Coulter and Chamberlain (1910). All of the cells formed are confined to the lower portions of the archegonia, which are very long and narrow, and decidedly pointed at the lower end. Walls do not appear until after sixteen free nuclei have been formed. The first walls appear on the division spindles after the four simultaneous nuclear divisions. Additional cells are soon formed so that at least thirty-two cells or probably more, are formed in the entire zygote before the suspensor elongates. As soon as the suspensor cells are distinguishable by their slight enlargement, we may recognize the embryo as organized into four groups of cells, some of them arranged more or less in tiers. This tier arrangement is not at all even as in Abietineae, and the further meristematic activity of the different regions which might be assigned to various tiers of cells make these stages much more irregular and variable in appearance than in such forms as *Pinus*.

The thirty-two cells are shown by Coker (1907) in his *figure 19*, and these are crowded into the base of this narrow cone-shaped archegonium with a single cell at the point, overlaid by the other cells, so that passing upward, one observes more and more cells somewhat unevenly placed, but all crowded into the lower third or two-fifths of the archegonium.

The large cell at the bottom of the archegonium forms a penetrating cap which appears to protect the primary embryo, much as a root cap protects an advancing root tip. The several adjacent cells above this, below the mass of embryonic meristem also remain large and inactive and are to be included with this lower one, as a group of cells forming the cap.

The next group of cells above the deciduous cap is a very active mass of cells which become small on account of their meristematic activity and are somewhat irregular in their arrangement. These cells which ultimately contribute the primary embryo can therefore not be spoken of strictly as a tier of cells. Above these is a third more even tier of from five to seven or more cells which form the primary suspensor and can be recognized as soon as these cells begin to elongate, while a fourth group of cells above the suspensor tier is composed of cells which are soon actively dividing, constituting the "rosette" cells. From all of the four accounts of the proembryo we have no evidence of the existence of a tier of free nuclei above this rosette group, another point wherein the proembryo of *Cephalotaxus* differs from that of Abietineae. In a sense the rosette cells of *Cephalotaxus* are therefore not strictly homologous to the rosette cells of the pine embryo, but since these are the uppermost walled cells in both cases above which the basal plate appears, we will speak of them as the rosette cells in this discussion.

DESCRIPTION OF OBSERVED STAGES

The present investigation begins with young embryos of the type shown in FIG. 1. Here we see two neighboring embryo systems coming from adjacent archegonia. A few stages younger than these (before suspensor elongation) were found, but could not be reproduced satisfactorily by photography and were therefore omitted.

In FIG. 1, the cap cells may be seen on each of the primary embryos. Above these are the groups of meristematic cells which give rise to the embryos proper, and above these are the primary suspensors which are made up of five to eight collateral elongating cells. A group of rosette cells is shown above this, and the plug of opaque substance which forms in the egg above the embryo is present in the zygote shown at the left. This probably corresponds to the basal plate of Abietineae. In the embryo to the right, this was lost in dissection.

The cavity of the gametophyte, into which the primary embryos are pushed by their suspensors, is formed by the corrosive action of enzymes which are secreted from some or all parts of the embryo. While mechanical action of the suspensors keeps the several embryos pushed forward as far as possible

in this cavity, the digestion actually precedes this mechanical action. Lawson (1907) describes a clear space in advance of the embryo tip, and in a study of the pine and other conifers, it has appeared certain to the writer that the suspensor does not force the embryo in a crushing manner through the firm living gametophyte tissue. Since these studies were made from dissections, no such sectional views as those mentioned by Lawson were observed. From the ease with which the embryos were removed there was very little or no evidence which would indicate that the suspensors were mechanically pushing their embryos forward faster than the digestive action of the enzymes opened up the cavity occupied by the embryos. Doubtless they crush aside the remains of collapsed cells after the enzymatic action has rendered the gametophyte tissue spongy.

The primary suspensors are at first straight and rather stiff, but in time they become coiled and twisted. They continue to elongate without the addition of a secondary suspensor in the form of embryonal tubes, until they have reached a great length, those in FIG. 4 measuring over 7 mm. long, counting the twists. Thus the embryonic cell group is pushed forward far into the gametophyte tissue, and during the stages represented between FIGS. 1 and 5, the large cap cell is usually lost. According to Lawson, it is lost very early in the succeeding stages. FIG. 4 represents the embryo systems from two adjacent archegonia, which had a very even start and developed side by side. It may be seen in FIG. 5, that the larger of the two primary embryos has lost its largest cap cell while the smaller still retains all of the cap. This is probably a late stage in which to find this organ present. In FIG. 3, an embryo is shown probably without its largest cap cell (though this may have been loosely attached and lost in the dissection). It is interesting to note that in the writer's earlier stages (FIGS. 1 and 3) the nuclei of these suspensor cells, together with a dense mass of cytoplasm, are situated in the lower end of the suspensor, i. e., near the mass of embryonic cells, but by the time the stage of FIG. 5 is reached they are usually not found. This position of the nucleus was also observed by the writer in practically all conifer embryos which he has had occasion to examine.

It is especially interesting to note that several embryos from different eggs are engaged in an embryonic "struggle for exist-

ence" and the suspensor is the weapon by which the adversaries are overcome. As the embryos grow longer (FIGS. 7–8), they give off a massive suspensor of embryonal tubes as in other conifers. These originate from the cells of the primary embryo nearest the older portions of the suspensor. These successive waves of elongating cells sometimes become entangled with their adversaries and sooner or later the vanquished are pushed back to the archegonial region until only one, the successful embryo, occupies the favorable position, which insures its survival as the embryo of the seed. Embryonic Selection is a special form of Developmental Selection and its significance in evolution has been outlined elsewhere (Buchholz, 1922).

THE EMBRYOS DERIVED FROM THE ROSETTE REGION

An outstanding new item in the present investigation is the demonstration that usually other embryos are derived from the rosette region. The group of rosette cells (upper part of FIGS. 1, 2, and 4) are all meristematic, and when followed through later stages (FIGS. 7–9) become active, developing into embryos. They are devoid of a primary suspensor, but soon become many-celled and of good size, sending out the embryonal tubes of a secondary suspensor (FIGS. 7–9), thus demonstrating that they are in the nature of real embryos. Their orientation is variable and probably they grow out in any direction. They do not possess the deciduous cap cells found in the primary embryo.

It is clear that in *Cephalotaxus* each zygote gives rise to several rosette embryos in addition to the primary embryo, which demonstrates that we are dealing with a compound embryo, that we have here a partially suppressed form of cleavage polyembryony.

FIG. 8 shows an embryo which is double. There is one large embryo and beside it a small one, as if to indicate an instance of cleavage polyembryony in the primary embryo. It is more probable that these are the embryos of two archegonia, as if FIG. 5 had developed to a later stage. Of course, it is not impossible that the main embryo may divide, but aside from FIG. 9, no apparent evidences of cleavage in the primary embryo have come to the writer's notice in the course of these studies, and it seems certain that cleavage of the primary zygote is at least not frequent. From the external appearances of the embryos

there is no evidence for the existence of an apical cell at any stage in the primary embryo, and there is also no external evidence of an apical cell in the rosette embryos.

Sections of later stages in the primary embryo are shown in FIGS. 10 to 12. The evidence which we have here indicates that the stem tip meristem is the last region of the embryo to become organized. In the Pine, the stem tip appears as a slight swelling before there is any evidence of the cotyledons, and while it does not develop further, it remains as a dome-shaped protuberance from this stage on. FIG. 10 shows the plerome, periblem, and dermatogen all organized. The latter extends well down the sides of the root cap. In the pine, the plerome of the root tip is the first region to differentiate and the dermatogen is not definite until much later. The cotyledon primordia are organized, and the plerome strands are beginning to extend into the cotyledons. The periblem is indicated between the dermatogen and plerome but is not sharply differentiated at the point where it joins the root cap, and the root cap further merges insensibly with the suspensor.

In the next stage observed (FIG. 11), the periblem is becoming more distinctly differentiated from the suspensor, but its separation is not sharp even in FIG. 12. The epidermis in FIG. 12 may be seen to extend well down the sides of the root cap to the suspensor region and it appears that the periblem and root cap are a continuous tissue of cells which merely become larger and more vacuolated as one passes from the plerome to the suspensor region. This entire region of the embryo is short compared with that of the pine embryo, where the periblem of the root tip and the root cap constitute nearly half of the length of the embryo at the time when the seed is ready to be shed.

The triangular mass of substance above the flat stem tip between the cotyledons (FIGS. 11, 12) is composed of a mass of undigested cell walls which have accumulated from the disintegrated cells of the surrounding gametophyte tissue. In the embryo of FIG. 10, a similar plug of some kind was removed with difficulty during the dissection. This object was found in all the embryos which were sectioned, but it is in no way derived from embryonic tissue.

The largest embryos which were sectioned showed a hollow pith cavity in the middle of the plerome region as indicated in

FIG. 12. This was found in all other embryos of this size. It is evidently formed by a breaking down of the cells of the plerome, and is accompanied by a swelling of the central region of the embryo. The embryo chosen for illustration in FIG. 12 did not show the swelling as much as some others. About twenty of these later embryos which were removed from the ovules all proved to possess two cotyledons.

FIG. 11. Section of older embryo of *Cephalotaxus* showing cotyledons half grown, periblem sharply differentiated from plerome and less distinctly from root cap. The stem tip is still flat. A plug of crushed cells derived from the disintegrated gametophyte tissues, lodged above the embryo body between the cotyledons, is not a part of the embryo. × 18.

FIG. 12. Embryo of seed in a stage nearly fully developed, but in which the stem tip is still flat and undifferentiated. Periblem has become more sharply differentiated from plerome, and the dermatogen extends distinctly over root cap to the region of the suspensor. A pith cavity of broken down cells has appeared in the center of the plerome. × 18.

Discussion

Previous investigators have compared the proembryo to that of the pine. There are, however, many points of difference and the homology between the embryogeny of *Cephalotaxus* and that of *Pinus* is not so evident from appearances. The first walls of the pine proembryo come in after eight free nuclei are formed, while in *Cephalotaxus* there are sixteen free nuclei before the cell walls appear. The terminal four cells in the pine proembryo function actively as apical cells for the respective four embryos while in *Cephalotaxus* the lowest cell which corresponds to the apical cell of the primary embryo in position is a single cell. It enlarges and becomes the terminal cell of the group constituting the cap. Finally we have a proembryo of many cells rather than one of sixteen cells at the time when the suspensors begin to elongate, and there appears to be no apical cell stage at all in the *Cephalotaxus* embryo.

The writer feels convinced from this study that the key to the real homologies between the parts of the embryo of *Cephalotaxus* and those of pines and other conifers lies in its cleavage polyembryony.

If embryo initials are represented by the proembryo at the time when the first walls are formed, then *Cephalotaxus*, which has walls organized around all of its nuclei when walls first appear, represents sixteen potential embryo initials, If any of the subsequent divisions represent still further organization of greater cleavage polyembryony there may be even more, as is probably the case in *Sciadopitys* (Buchholz, 1920). Of course, *Cephalotaxus* has practically eliminated the cleavage polyembryony for the primary embryo; at least the writer found only one instance (FIG. 8) where two or more embryos could have been formed by division of the meristematic mass of cells next to the cap cell, and this one is extremely doubtful. The writer believes that the organization of this cap was partially, if not largely instrumental in the elimination of cleavage polyembryony during the early stages. Lawson (1907) states that "the early meristematic activity of the (primary) embryo may explain the necessity for the development of the terminal cells into a penetrating cap. This explanation finds support in the fact that the cap cells are thrown off very soon after the suspensors are developed." At any rate, if excessive cleavage polyembryony existed

in forms ancestral to *Cephalotaxus*, the cap was probably not then present, and its origin may have been associated with the suppression of both cleavage polyembryony and the apical-cell stage. The present protective function as an organ of penetration or even as a secreting organ is not denied in assigning this rôle to the cap cells, but the writer is also convinced that this is not a very essential organ, for embryos frequently shed their caps in early stages and most conifers have no such organ at any stage of their embryogeny.

The cap cells are therefore not homologous with anything in the embryo of the pine unless they represent one or more abortive embryo initials. The mass of cells of the embryo proper above the cap cells represents a large number of the embryo units (embryo initials) corresponding to the four which separate in *Pinus* and the probably larger number found in other conifers such as *Sciadopitys* (Buchholz, 1920). The suspensor cells may represent the first cells cut off by some of the embryo initials of this group, or more probably they represent still other embryo initials which elongate. In the pine, the rosette cells which are unquestionably embryo initials sometimes elongate as suspensor cells in such a manner. That the rosette cells are embryo initials is fully demonstrated.

Though the group of rosette cells above the suspensors were observed by others, they have apparently not been studied, at least their destiny as embryo forming cells was not suspected. Strasburger (1879), Coker (1907), and Lawson (1907) mention them as a group of rosette cells, but followed them no further. Coker agrees with Strasburger's observation in noting these rosette cells, probably of the stage shown in FIG. 2.

While Strasburger figured the early stages of these rosette embryos without comment, Coker and Lawson both figured small secondary embryos above the primary one in an older stage, but regarded them as the result of later fertilization of archegonia, which is also possible in the sections which they figured. Lawson considered them as possible cases of embryos budding from "the main group of embryo cells and the formation of small secondary embryos. These however, are not frequently found. As a rule there is but one embryo formed from a single archegonium." It is likely that both Lawson and Coker were dealing with some of these secondary or rosette embryos.

The existence of these active rosette embryos associates *Cephalotaxus* with the group of conifers which have a modified form of cleavage polyembryony. It is from the behavior of these rosette cells that we obtain a definite clew to the polyembryonic history of *Cephalotaxus*. Being uppermost in their position these are probably homologous with the rosette embryos of the pine if we disregard the free nuclei above the rosette cells in *Abietineae*. When cleavage polyembryony was suppressed in the lower portion of the embryo system, this did not do away with cleavage polyembryony in the rosette group. At least we are better able to make comparisons between the embryo of *Cephalotaxus* and those of Abietineae, and other conifers, when we consider the nature of these secondary rosette embryos in the light of a polyembryonic origin.

This also adds to our evidence for the view that cleavage polyembryony is a condition through which the Coniferales have passed in their evolution, for certainly *Cephalotaxus* supplies us with satisfactory ontogenetic evidence of this kind. A further discussion of this very fascinating problem of the origin and evolution of cleavage polyembryony will be treated by the writer in another paper.* It may be stated here that the origin of this condition represents an episode in the evolution of conifers, having to do with the beginnings of siphonogamy in the origin of seeds.

Summary

We have, in *Cephalotaxus*, an embryo which is derived from a condition of cleavage polyembryony. Most, if not all, of the early cells of the proembryo are to be regarded as embryo initials. Of these, the terminal cells do not form embryos but go to form the cap instead. The next group of proembryonic cells above the cap combine to form a single embryo. The primary suspensor is formed from a tier of cells above these, and becomes very long, while the uppermost tier, the rosette cells, are the only ones which retain fully their separate embryo-forming capacity, usually giving rise to several rosette embryos. No certain evidence of cleavage of the primary embryo was observed.

* Concerning the origin of cleavage polyembryony in conifers. To appear in Botanical Gazette.

In the later embryo the stem tip is differentiated late, long after the dermatogen, periblem, plerome, root cap, and cotyledons have become fully organized,—in a stage later than any in the figures shown in this treatise. The dermatogen appears early and extends down over the root cap to the region of the suspensor. A cavity is formed in the later stages by a breaking down of cells in the middle of the plerome region. The embryos usually have two cotyledons.

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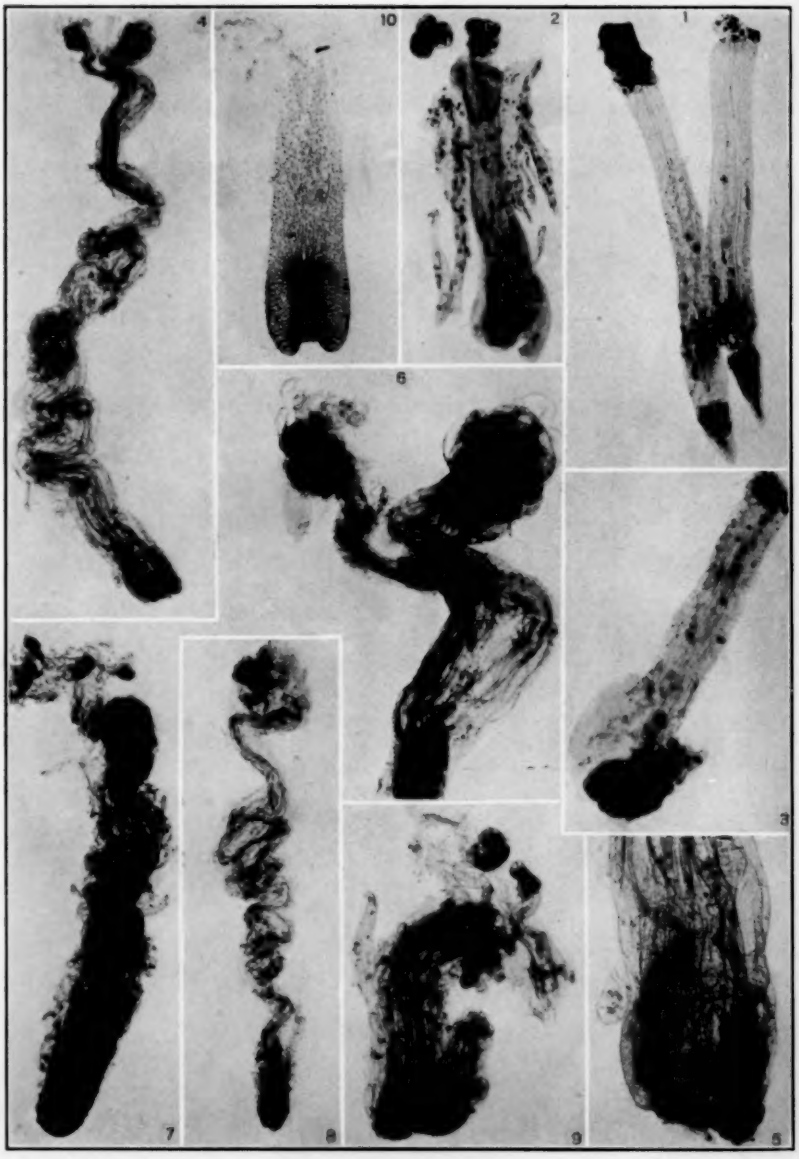

CEPHALOTAXUS FORTUNEI

Description of plate 10

FIG. 1. Two embryos from neighboring archegonia dissected out of gametophyte and mounted. Beginning below, they show the cap cells, next a group of cells forming primary embryo, above these the elongating suspensor cells, and above the suspensors a group of cells forming the rosette embryo. In the embryo to the left is a mass of deeply staining substance found above the rosette in the archegonium (which was removed by the dissection in the embryo to the right). This probably corresponds to the basal plate of Abietineae. × 56.

FIG. 2. Dissection of slightly younger stage than FIG. 1, showing rosette embryos slightly separated by dissection. Suspensor cells have their lower ends slightly enlarged and nearly all of the nuclei are found here. The primary embryo with its cap cells was lost in the dissection. × 56.

FIG. 3. Embryo of the same age as FIG. 2 with primary embryo in position but with the terminal member of the group of cap cells missing, probably lost in dissection. × 56.

FIG. 4. Embryos of two adjoining archegonia with suspensors fully elongated. The rosette embryos are developing from the rosette above and are shown enlarged in FIG. 6. Embryonal tubes have not begun to elongate to form the secondary portion of the suspensor. × 18.

FIG. 5. More detailed view of primary embryos of FIG. 4. The embryo to the left still possesses the terminal cap cell, while the larger one to the right has discarded its largest cap cell. × 56.

FIG. 6. More detailed view of rosette embryos of FIG. 4, showing the embryonal tubes forming a secondary suspensor. No primary suspensor is developed for the rosette embryos. × 56.

FIG. 7. Older embryo with massive secondary suspensor well developed and with older group of five rosette embryos above, in various stages. × 10.

FIG. 8. Embryos younger than FIG. 7, with apparently two primary embryos formed side by side (?) and a single very irregular rosette embryo above. The rosette embryos shown in FIG. 9 belong with these. × 29.

FIG. 9. Part of the rosette embryo group dissected away from the embryo system of FIG. 8. Note absence of cap and primary suspensor, the lobed and irregular character of the rosette embryos which have developed very unequally. × 56.

FIG. 10. Section of embryo of *Cephalotaxus* with body regions organized and cotyledons developing. Stem tip is unorganized, plerome is distinguishable from periblem, but periblem, root cap and suspensor merge into each other. Dermatogen appears to extend down over root cap. × 18.

Studies on the flora of northern South America—VI*

H. A. Gleason

New or noteworthy species of Melastomataceae

Generic characters seem to be sharply marked in the majority of melastomataceous genera. The chief exceptions are found in the tribe Miconieae, where genera are distinguished largely on the position of the inflorescence and the shape of the petals. Here also are found many cases of remarkable similarity in habit between species of different genera, such as *Leandra* and *Miconia*, and it becomes extremely difficult or even impossible to place some immature or fruiting specimens into a genus with confidence. Within the genus *Miconia*, with well over six hundred species, the eleven sections are not always easily differentiated. They are characterized by the shape and dehiscence of the anthers, and there seems to be an almost continuous series of anther-shapes between the subulate, attenuate anthers of *Tamonea* and the linear ones of *Eumiconia*, and from the latter to the oblong, small-pored anthers of *Amblyarrhena*, and on to the obovate, large-pored anthers of *Cremanium*. Sections *Jucunda*, *Tamonea*, and *Adenodesma* are very close, as are also *Cremanium* and *Chaenopleura*. I have found no good evidence to warrant any segregation of the genus.

It is in most cases absolutely necessary to soften flowers by boiling and to make careful dissections in order to determine with any degree of accuracy the structure of the stamens. This has been done for all of the species described below, excepting a very few in which the stamens are not mentioned. All measurements of stamens, styles, seeds, and other small floral organs have been made with an eye-piece micrometer from boiled material, and have always been taken from the type specimen only.

Within a single flower, deviations in measurements from the stated dimensions are negligible, and rarely amount to as much as 5 per cent. Greater variations are found between different flowers of the same plant, or between different plants of the same

* Contributions from the New York Botanical Garden, no. 274.

species. These are probably due chiefly to age, but we do not know anything about the relative amount of elongation that takes place in the flower of a melastome during anthesis. It follows, therefore, that the measurements here given, although accurate in themselves, might not be repeated precisely in any other dissection.

There are similar variations in size in the leaves of any herbarium specimen. The measurements given here are taken, when expressed without any variation, from the largest leaf, or, when a range of size is indicated, from typical foliage leaves situated below the inflorescence. It is worthy of note that few sheets are examined in which the size of the leaves is as great as that given by Cogniaux, who seems to have allowed for an increase in size of those leaves farther down the stem and consequently not collected in a specimen of ordinary size.

Pterogastra glabra n. sp. Herbaceous, apparently erect, sparingly branched above; stems green, slender, narrowly 4-alate, glabrous, or very rarely with a few minute appressed hairs on the wings, sparsely setose at the nodes; petioles glabrous, 2–3 mm. long; leaf-blades firm, oblong-lanceolate, 30–45 mm. long, 5–9 mm. wide, acute, often minutely aristate, minutely crenulate and setose-ciliate with erect hairs nearly 1 mm. long, acute at base, bright green and glabrous on both sides, 3-nerved, the veinlets very obscure; flowers 5-merous, few, in loose, open, bracteate cymes terminating the stem and branches; peduncles narrowly 4-alate or merely quadrangular; bracts subulate, 2 mm. long, ciliate; pedicels 2 mm. long, glabrous; hypanthium bright green, campanulate, 6–7 mm. long, prominently 10-nerved, glabrous, or the 5 nerves opposite the sepals somewhat stronger and rarely appressed-spinulose; sepals green, herbaceous, triangular-lanceolate, 6 mm. long, 2 mm. wide at base, acute, aristate with a seta 1 mm. long, prominently setose-ciliate, obscurely reticulate; petals apparently blue, broadly obovate, about 13 mm. long, finely ciliate; anthers linear-subulate, yellow, 10 or 6 mm. long, arcuate retrorsely, opening by a minute terminal pore; connective slender, prolonged 2 or 0.5 mm.; bearing 2 stout, blunt, rounded, anterior appendages 0.5–0.7 mm. long; filaments slender, 6 or 5 mm. long; ovary ellipsoid, 4 mm. long, with a terminal circle of slender, glandless setae 0.7 mm. long; style terete, 3.5 mm. long. FIG. 1.

Type, *Pittier 10,577*, collected in wet savannas, Mene Grande, State of Zulia, Venezuela, 28 Oct. 1922, and deposited in the herbarium of the New York Botanical Garden. It is

closely related to *P. divaricata* (Bonpl.) Naud., but differs in its glabrous leaf-surface, essentially glabrous stem, almost wingless, usually glabrous hypanthium, and shorter ovarial setae.

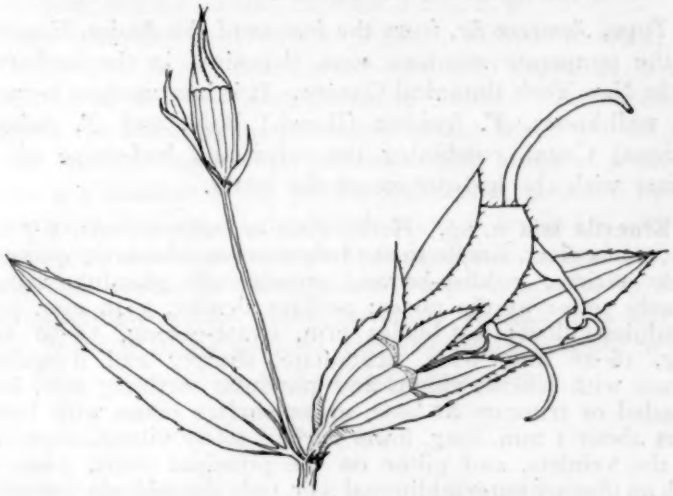

FIG. 1. Pterogastra glabra Gleason, flower, bud, and leaves, × 2.

TIBOUCHINA GRACILIS (Bonpl.) Cogn. Several varieties of this widely distributed species have been distinguished, most of which seem to depend chiefly on eccentricities in habit alone. Our series of specimens from Colombia includes two forms which differ not only in habit, but also in the structure of the anther. In *Rusby & Pennell 1051*, collected east of Neiva, Dept. Huila, even the lowest cymes are on peduncles less than 1 cm. long, the upper cymes are closely approximate, and the pubescence of the leaves and stem is unusually long. The connective of the anther is not prolonged, but bears two minute gibbosities on the posterior side and a bilobed anterior appendage. The end of the filament is directly under the base of the anther. In our other specimens, the lower cymes are borne on conspicuous peduncles, the upper cymes are well separated, and the pubescence is less conspicuous. The connective is prolonged basally for nearly 1 mm. at right angles, and bears the usual 2-lobed anterior appendage and also a 2-lobed posterior, deflexed appendage about 0.2 mm. long. The end of the filament is quite out of line with the anther.

Tibouchina lepidota intermedia n. var. Leaves ovate-elliptic, 8–10 cm. long, 4–4.5 cm. wide, strongly bullate-muricate on the upper surface, beneath foveolate, densely paleaceous on the veins, pubescent on the surface.

Type, *Jameson 87*, from the forests of the Andes, Ecuador, in the temperate montane zone, deposited in the herbarium of the New York Botanical Garden. It is intermediate between the well-known *T. lepidota* (Bonpl.) Baill. and *T. paleacea* (Triana) Cogn., combining the calyx and leaf-shape of the former with the leaf-surface of the latter.

Ernestia lata n. sp. Herbaceous or suffrutescent, 5–7 dm. tall; stems erect, simple to the inflorescence, obscurely 4-angled, finely striate, reddish-brown, prominently glandular-villous, densely setose at the nodes; petioles slender, 5–10 mm. long, glandular-villous; leaf-blades firm, ovate-oblong, 35–50 mm. long, 16–28 mm. wide, acuminate, sharply and irregularly serrate with salient, villous and glandular teeth 0.5 mm. long, rounded or truncate at base, upper surface pilose with brown hairs about 1 mm. long, lower surface softly villous, especially on the veinlets, and pilose on the principal veins, 5-nerved, with an obscure outer additional pair, only the midvein impressed above, the veinlets reticulate beneath; upper leaves gradually reduced in size; inflorescence a terminal, nearly leafless panicle 1–2 dm. long, with slender, spreading branches, glandular-villous like the stem; bracts linear-subulate, 2–5 mm. long; pedicels mostly 2–5 mm. long; hypanthium broadly campanulate, 2.7 mm. long by 2.5 mm. in diameter, obscurely 4-ribbed, glandular-villous with spreading hairs 1 mm. long; calyx prolonged 0.3 mm. beyond the staminal torus; sepals triangular-subulate, 4.5 mm. long, acuminate to an aristate tip, entire, widened at the base and meeting in broadly rounded sinuses, the total width 2 mm., sparsely glandular-villous, 3-nerved; petals 4, broadly round-ovate, 6 mm. long, 5 mm. wide, obtuse, truncate at base, glabrous, sparsely setose-ciliolate at the apex; long stamens 4, opposite the sepals: anthers lance-subulate, 2.7–3 mm. long, 0.6 mm. wide at base, opening by an oblique introrse pore; connective slender, thickened below the anther and prolonged 2 mm.; anterior appendage reverse-triangular at base, 1.1 mm. long and wide, flat, prolonged into 2 erect, flat, lance-subulate, straight or flexuous, sparsely serrulate spurs 2.5 mm. long, 0.4 mm. wide at base, separated by a truncate sinus; posterior appendage a minute conic apiculum 0.1 mm. long; filaments brown, flat, straight, 2.0 mm. long, 0.4 mm. wide; short stamens 4, opposite the petals: anthers 2.7 mm. long; connective prolonged 1 mm. at base; anterior appendage narrowly triangular at base, prolonged into 2 linear-subulate, flat, contiguous spurs

3.3 mm. long; posterior appendage conic, 0.2 mm. long; filaments 1.6 mm. long; ovary ovoid-globose, sparsely setose on the upper half and densely glandular-setose at the summit; style filiform, 5 mm. long; stigma minute, capitate. FIG. 2, B.

Type collected by *Weiss & Schmidt*, without number, on the upper Rio Negro, Brazil, 1907–1908, and deposited in the herbarium of the New York Botanical Garden. The measurements of the floral organs are taken from a flower not yet expanded, and the stamens may be considerably longer at anthesis. *E. lata* is a member of the section *Euernestia*, as shown by its setose ovary, and is most closely related to *E. tenella* DC., which was collected in the same general region by Bonpland and by Spruce. *E. tenella* is distinguished from our species by narrower, sparingly serrate leaves, glandular on both sides and on longer petioles, longer pedicels, glabrous petals cuneate to the base, longer connectives, and different appendages.

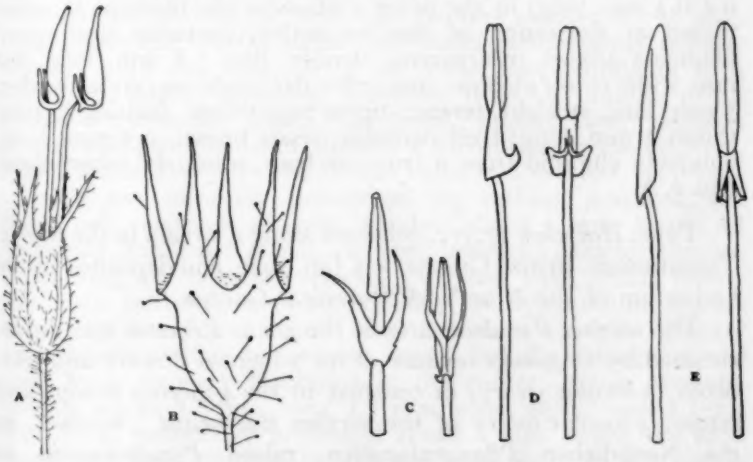

FIG. 2. A. Ernestia glandulosa Gleason. B. Ernestia lata Gleason, hypanthium; C. stamens. D, E. Copedesma nitens Gleason. × 5.

Ernestia glandulosa n. sp. Stem herbaceous, 6 dm. high, simple or nearly so, obscurely 4-angled, densely glandular-villous with purple-red hairs when young, becoming sparsely glandular in age, the internodes 3–7 cm. long; leaves of each pair similar; petioles slender, 15–25 mm. long, glandular-villous like the stem; leaf-blades thin and membranous, ovate-oblong, 50–85 mm. long, 20–35 mm. wide, long-acuminate, finely and sharply denticulate (teeth ascending, triangular, 0.2–0.6 mm. long,

about 15 per cm. of margin), rounded or subcordate at base, glandular-villous near the margin, upper surface dark green, villous with ascending hairs about 1 mm. long, lower surface dull green, pubescent with hairs about 0.5 mm. long and purple-villous along the veins, 7-nerved, the veinlets obscure, crooked; racemes 1–3-flowered, axillary, 1–2 cm. long; rhachis and pedicels glandular-villous, bracts subulate, 1 mm. long, setose-tipped; pedicels 3 mm. long, or 8 mm. long in fruit; flowers 4-merous; hypanthium ellipsoid, 3 mm. long, obscurely 8-ribbed, green, glandular-villous; sepals subulate, 2 mm. long, erect, persistent, ciliate and sparsely glandular-villous; petals apparently white, about 8 mm. long, glabrous; stamens similar in size but dimorphic; anthers linear-subulate, 2.8 mm. long, 0.5 wide at base, somewhat recurved at the apex, opening by a minute terminal pore; connective prolonged below the anther toward the anterior side for 0.5–0.7 mm. and terminated by 2 erect, filiform appendages 0.8–0.9 mm. long and nearly parallel to the anther; in 4 stamens the filament is articulated at the anterior end of the connective below a short, conic, descending, posterior appendage 0.2–0.3 mm. long; in the other 4 stamens the filament is articulated at the center of the connective, posterior spur none; filaments almost transparent, slender, flat, 3.8 mm. long, 0.2 mm. wide; ovary globose, free, trilocular, glabrous; style slender, 5 mm. long, straight, terete; stigma punctiform; fruiting hypanthium 4 mm. long; fruit capsular; seeds brown, 0.5 mm. long, obliquely ellipsoid from a truncate base, minutely tuberculate. FIG. 2, A.

Type, *Hitchcock 17,372*, collected along a stream in the forest, Tumatumari, British Guiana, 3–5 Jan. 1920, and deposited in the herbarium of the New York Botanical Garden.

The section *Pseudoernestia* of the genus *Ernestia* was differentiated by Cogniaux because of its 5-merous flowers and glabrous, 3-locular ovary, in contrast to the 4-merous flowers and setose, 4-locular ovary of the section *Euernestia*. Krasser, in the Natürlichen Pflanzenfamilien, raised *Pseudoernestia* to generic rank, chiefly, apparently, because of its glabrous ovary. *E. cordifolia* O. Berg., the only species of the section, is known, so far as I can discover, only by a single collection of Spruce on the Orinoco River. Our species differs from it in its small axillary racemes, narrower leaves, and 5-merous flowers. Since it combines the ovary of *Pseudoernestia* with the 5-merous flowers of *Euernestia*, it seems highly doubtful whether Krasser's separation is justifiable, and I have described it under the broader genus *Ernestia*.

Copedesma, n. gen.

Flowers 5-merous; hypanthium obconic; sepals broadly triangular, short, the exterior teeth none; petals obovate-oblong, rounded at the apex, glabrous; stamens dimorphic, uniform in size; anthers narrowly linear, opening by a minute terminal pore; filaments elongate, filiform, glabrous; connective much prolonged below the base of the anther-sacs, in some stamens widened posteriorly beyond the attachment of the filament into a flattened, truncate appendage, not appendaged anteriorly, in the others expanded anteriorly at the insertion of the filament into two broad, blunt, triangular, laterally spreading appendages, continuous around the apex of the filament into the broad, truncate, posterior appendage; ovary glabrous, radially ribbed; style filiform, elongate, glabrous; stigma capitate. Woody plants with dark green, glossy foliage, furfuraceous stems, and terminal, slender, stellate-tomentose panicles.

Copedesma nitens n. sp. Stems 12 dm. high, branched above, the branches terete or nearly so, closely furfuraceous-tomentulose, glabrescent with age; petioles slender, 10–18 mm. long, pubescent like the stems; leaf-blades membranous, oblong-elliptic, 9–14 cm. long, 3–5.5 cm. wide, long-acuminate to a blunt tip, entire, gradually narrowed from the middle to an obtuse or rounded base, dark-green, shining, and glabrous above, dull green beneath, densely tomentulose on the midvein, thinly furfuraceous on the lateral veins and veinlets, and very sparsely and minutely stellate on the surface, 5-nerved, the outer pair relatively obscure, veinlets about 5 mm. apart, all distinctly impressed above, elevated beneath; panicles slender, 7 cm. long, the branches remote, 5–10 mm. long; bracts linear-spatulate, stellate-tomentose, equaling the hypanthium; flowers sessile; hypanthium 3.2 mm. long, 3 mm. in diameter, densely and closely stellate-tomentose; sepals broadly triangular, 0.6 mm. long, strongly thickened in the center; petals obovate-oblong, white, 2.8 mm. long, 1.7 mm. wide; anther-sacs 2.1–2.4 mm. long; connective prolonged about 1 mm. below the sacs, stout and thick, gradually widened posteriorly to a truncate appendage 1.5 mm. wide, in some stamens bearing also two anterior, broadly conic, laterally spreading appendages, together 1 mm. wide; filaments very slender, glabrous, 4.7 mm. long; style 7.5 mm. long; stigma 0.5 mm. in diameter. FIG. 2, D, E.

Type, *La Cruz 1235*, collected at Tabla, Pomeroon District, British Guiana, 28 Sept. 1921, and deposited in the herbarium of the New York Botanical Garden. Other specimens of the same collection are in the Gray Herbarium, the National Herbarium, and the Philadelphia Academy of Sciences.

This remarkable plant, which unfortunately presents but few open flowers and no fruits, bears a strong superficial resemblance to *Miconia*, but the character of the stamens indicates at once that it is far removed from that genus, and probably in the general affinity of *Ernestia*, of the tribe Tibouchineae. Unlike most genera of that tribe, the anther-sacs, connective, and filament lie in the same line, the connective is unusually stout, in diameter equaling the anther-sacs and considerably exceeding the filament, and the stout appendages are not prolonged into a filiform spur. The general arrangement of these parts is shown far better by the accompanying figures than by description. In the absence of fruit and seeds, its tribal position must remain open to question, but a comparison with the numerous figures of other genera in Triana's monograph or in the Flora Brasiliensis will show its fundamental similarity to the Tibouchineae in anther-structure. The anthers are fragile and easily detached from the filaments: in each of the two flowers dissected most of the anthers were missing. It is probable that there are five of each kind, but that could not be confirmed, since the filaments are uniform and it can only be said that both kinds exist in each flower. The generic name is from two Greek roots signifying *sword-hilt* and *connective*.

Acisanthera glomerata n. sp. Herbaceous or suffruticose, probably decumbent at base; stems freely branched, sharply quadrangular or narrowly alate, glandular-villous with hairs about 0.5 mm. long; leaves sessile, obovate-spatulate, 7–20 mm. long, 3–6 mm. wide, obtuse, entire or rarely obscurely serrulate, cuneate from above the middle to the base, minutely hirtellous on both sides, glandular-ciliate, obscurely 3-nerved; cymes numerous, terminating the stem and branches, on peduncles 8–15 mm. long; bracts and bractlets sessile, broadly obovate, 4–8 mm. long, obtuse, prominently serrulate toward the apex, pubescent like the leaves; pedicels 1 mm. long or less; flowers 4-merous; hypanthium short-cylindric, 2.8 mm. long, conspicuously 8-nerved, glandular-villous; sepals erect, persistent, membranous, triangular, 3.7 mm. long, 2.5 mm. wide at the base, sharply acute and tipped with a glandular seta, sparsely glandular-villous, prominently 3-nerved and reticulately veined; large stamens 4: anthers oblong, straight, 1.5 mm. long, terminated by a straight, slender, terete rostrum 0.8 mm. long, opening by an introrse terminal pore; connective slender, prolonged 1 mm. at base, sharply introrse for 0.5 mm., and ending in 2 anterior, erect, filiform appendages 1 mm. long, the posterior

side entire or minutely gibbous; filaments flattened, filiform, 2.8 mm. long; small stamens 4: anthers oblong, 1.1 mm. long, terminated by a slender rostrum 0.4 mm. long, the connective prolonged at base 0.4 mm., straight, not appendaged, the filaments slender, 2.8 mm. long; ovary broadly ellipsoid, glabrous, minutely 4-lobed at the summit, 4-locular; style filiform, 4 mm. long, the minute stigma capitate; fruiting hypanthium 3 mm. long; capsule 2-valved, 4-locular, glabrous; seeds brown, 0.5 mm. long, cochleate. FIG. 3, A.

Type, *Samuels 424*, collected in the Forest of Zandery, near Parimaribo, Surinam, 3 July 1916, and deposited in the herbarium of the New York Botanical Garden. Although it has the quadrilocular ovary of Cogniaux's section *Dichaetandra*, it seems to be more closely related in structure and habit to *A. Eoisseriana* Cogn. and *A. hedyotidea* (Presl.) Triana, which have a bilocular ovary, shorter styles, ovate leaves, and shorter sepals separated by conspicuous setae in the sinuses.

Monochaetum rotundifolium Cogniaux, n. sp. Stem shrubby, about 1 m. high, freely branched above, prominently 4-angled, densely pubescent or subtomentose with straight, brown, plumose hairs 0.3–0.5 mm. long; petioles stout, spreading, pubescent like the stem; leaf-blades firm, broadly ovate to subrotund, entire, prominently ciliate, subacute, rounded or somewhat emarginate at base, 7–9-pli-nerved, the outer pair of veins always obscure, upper surface dull green, densely strigose with stout, somewhat plumose hairs papillose at base, lower surface densely and softly pubescent with brown plumose hairs along the veins and gray hairs on the surface; flowers 4-merous, in terminal cymules of 3–7, the peduncles 5–10 mm. long, pubescent like the stem, the pedicels 3–6 mm. long, purple, the pubescence more appressed, decreasing distally, and somewhat glandular; hypanthium purple, ellipsoid-campanulate, 5 mm. long, sparsely appressed-strigose and glandular, pilose at the summit, especially at the sepalar sinuses; sepals ovate-triangular, 3.5 mm. long, acuminate, sparsely setulose, minutely ciliate; petals broadly elliptic, apparently about 10 mm. long, ciliolate; large anthers lance-subulate, 7 mm. long, conspicuously recurved above the middle, opening by a single terminal dorsal pore, on filaments 6 mm. long, the posterior appendage 2.2 mm. long, expanded above into a broadly lanceolate, somewhat dilated tip; small anthers linear-subulate, 4.5 mm. long, nearly straight, on filaments 7 mm. long, the appendage flat, elliptic-lanceolate, 2 mm. long; ovary nearly free, obscurely 4-angled, pubescent above, minutely 4-lobed and setose at the summit; style slender, 9 mm. long, decurved near the apex; stigma punctiform. FIG. 3, D.

Type, *Smith 1851*, collected on San Lorenzo Ridge, Santa Marta, Colombia, alt. 6000–7000 ft., 27 Feb. 1899, and deposited in the herbarium of the New York Botanical Garden. The sheet exhibits two specimens, differing only in the size of the leaves, and is accompanied by the collector's note: "A shrub 3–4 ft. Common on open ridges, 6000–7500 ft. Below 6500 ft. the leaves are larger, but the plants do not otherwise differ. Flowers observed Jan.–March. Petals rosy. The specimens are from the San Lorenzo Ridge, Feb. 27; the small-leaved one collected at about 7000 ft., the other at about 6000 ft." On the large-leaved plant, the length of the petiole is fairly uniform at 7–9 mm.; the size of the blade varies from 30 by 24 mm. for the largest to 10 by 8 mm. for those subtending the cymules. On the small-leaved specimen, which is to be considered the type, should differentiation be necessary, the petioles rarely exceed 5 mm. and the blades vary from 15 by 11 mm. down to 7 by 5 mm.

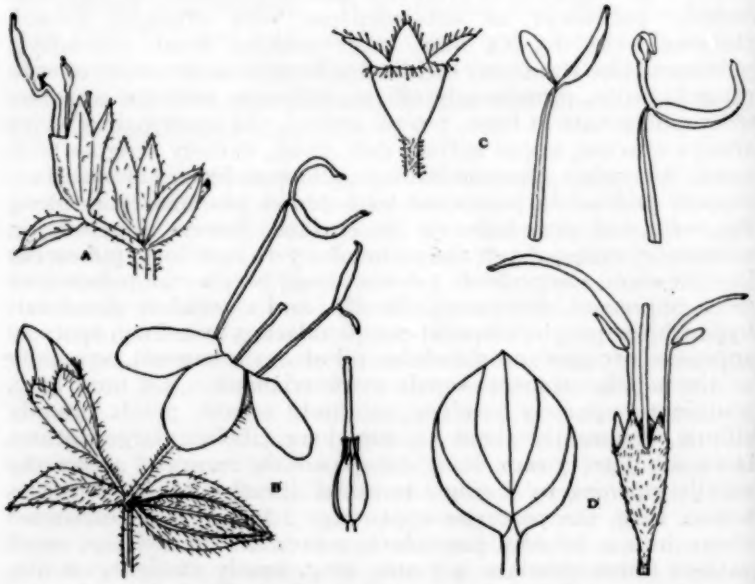

FIG. 3. A. Acisanthera glomerata Gleason, × 2.5. B. Monochaetum villosum Gleason, × 2; ovary and style, × 2. C. Monochaetum coronatum Gleason, hypanthium and stamens, × 2.5. D. Monochaetum rotundifolium Cogn., × 2.

Cogniaux, who examined the plant, noted that it represented an undescribed species, suggested the name here used, and indicated its close affinity to *M. stellulatum* Naud. Our species differs in its smaller and proportionately broader leaves, in its much larger anthers with proportionately smaller appendages, its longer sepals, and its colored flowers.

Monochaetum villosum n. sp. Stem shrubby, freely branched above, minutely 4-angled, villous with spreading or somewhat deflexed, slender, purple hairs 1.5–2 mm. long, the nodes densely villous; petioles slender, about 5 mm. long, villous like the stem; leaf-blades firm, ovate, entire, subacuminate to an acute apex, rounded or subcordate at base, 5-pli-nerved, the veins obscure above, somewhat conspicuous beneath, the veinlets obscure, upper surface softly villous with spreading, slender, pale hairs, slightly enlarged toward their base, about 2 mm. long, set about 0.3 mm. apart, lower surface more sparsely villous with similar but shorter hairs; flowers 4-merous, in cymules of 3, or rarely single, terminating the upper branchlets, on pedicels 2–4 mm. long; hypanthium purplish-green, narrowly campanulate or obconic, 5 mm. long, tapering to the base, faintly 8-ribbed, sparsely villous with straight, slender, spreading purple hairs, about 1 mm. long, set 0.3–0.5 mm. apart, and frequently glandular; sepals membranous, ovate-triangular, 1.8 mm. long and wide, barely acute, sparsely setose on the back like the hypanthium, strongly ciliate with curved hairs 0.8 mm. long; petals white, obovate, 8 mm. long, minutely ciliate; stamens dimorphic; anthers linear-subulate, opening by a single dorsal terminal pore, connective not prolonged anteriorly, prominently appendaged on the posterior side; larger anthers strongly curved, 6.5 mm. long, the appendages 4 mm. long, with 2 longitudinal ridges at the base and 3 at the somewhat expanded apex, the filaments 5.7 mm. long, flat, 0.7 mm. wide at base, tapering slightly to the summit; smaller anthers nearly straight, 4.3 mm. long, the appendages strongly flattened, spatulate, 2.7 mm. long, 0.6 mm. wide, the filaments 7.3 mm. long, flattened below, terete above; ovary free, 4-lobed, 4-locular, glabrous below, the summit minutely 4-lobed, each lobe tipped with 2 or 3 glandular setae 0.7–1 mm. long; style glabrous, subulate, declined, 6.5 mm. long; stigma punctiform. FIG. 3, B.

Type, *Rusby & Pennell 804*, collected in forest at "Balsillas," on the Rio Balsillas, Dept. Huila, Colombia, alt. 2100–2200 m., 3–5 Aug. 1917, and deposited in the herbarium of the New York Botanical Garden. The largest leaves are 18–22 mm. long by 11–14 mm. wide; the upper leaves are reduced and those subtending the cymules are barely 5 mm. long. The species is a

member of the section *Eumonochaetum*, with persistent sepals, and is most closely related to the Peruvian *M. dicrananthe rum* (R. & P.) Naud.

Monochaetum coronatum n. sp. Stem shrubby, freely branched above, obscurely 4-angled, finely and persistently glandular-pubescent with spreading hairs mostly 0.2–0.4 mm. long, densely hirsute at the nodes; petioles stout, 3–5 (rarely 10) mm. long, densely glandular-hirsute; leaf-blades thin, broadly ovate, entire, glandular-ciliate, broadly acute, rounded at the base, 5–7-pli-nerved, upper surface conspicuously but sparsely setose with appressed or ascending, yellowish, non-glandular hairs 0.6–1 mm. long, lower surface softly pubescent with spreading, slender, white hairs; flowers 4-merous, in terminal cymules of 3–5, on densely glandular pedicels 3–7 mm. long; hypanthium purplish, campanulate, 5 mm. long by 3.7 mm. in diameter, obscurely 4-angled, glabrous on the sides, setose at the summit with sparse, erect or ascending, glandular hairs nearly or quite 1 mm. long; sepals triangular, 2.8–3 mm. long, 2.5 mm. wide, acute, sparsely glandular-setose on the back, conspicuously glandular-ciliate, glabrous within; petals white, broadly elliptic, 1 cm. long, obtuse, glabrous, very sparsely glandular-ciliate at the apex; filaments of the large stamens flat, nearly 6 mm. long, of the small stamens similar, 8 mm. long; anthers of the large stamens lance-subulate, 6.3 mm. long, strongly recurved, with a single terminal dorsal pore, the basal posterior appendage 5 mm. long, its basal half stout, 2-ribbed on the dorsal side, the distal half more or less inflated, ovate-lanceolate, blunt, strongly bent or recurved near the apex; anthers of the small stamens linear-subulate, nearly straight, 4.8 mm. long, the appendage flat, 3.3 mm. long, the distal two-thirds expanded into an elliptic obtuse tip 0.8 mm. wide; ovary nearly free, obscurely 4-lobed, minutely 4-lobed at the summit, glabrous; style slender, 7 mm. long, glabrous, decurved near the apex; stigma punctiform. FIG. 3, C.

Type, *Pennell 2449*, collected in a clearing, 2–4 miles south of Sibate, Dept. Cundimarca, Colombia, alt. 2800–2900 m., 13–15 Oct. 1917, and deposited in the herbarium of the New York Botanical Garden. The largest leaves measure 35 by 21 mm., the upper are gradually reduced in size, and those subtending the cymules are only 10–15 mm. long. *M. coronatum* is most closely related to *M. Jahnni* Pittier, a Venezuelan species which also has a glabrous hypanthium surmounted by glandular sepals. Our species differs in its denser glandular pubescence, glandular-ciliate leaves, glandular pedicels, longer, acute sepals, larger, white petals, and much larger anthers.

Rhynchanthera microphylla n. sp. Stem shrubby, prominently 4-angled and swollen at the nodes, freely branching above, thinly pubescent above with spreading non-glandular hairs 0.1–0.2 mm. long, becoming glabrate with age, the internodes 1–2 cm. long; petioles flattened, 2–6 mm. long, pubescent like the stem; leaf-blades firm, oblong-ovate, 9–20 mm. long, 5–11 mm. wide, minutely setulose-serrulate, acute, broadly obtuse to subrotund at base, 5-nerved, upper surface thinly strigose with yellow hairs 0.2–0.3 mm. long, the lower thinly pubescent with spreading hairs of the same size; flowers 5-merous, in terminal cymules of 1–3, the villous pedicels 1 mm. long; hypanthium 6 mm. long, cylindric below, expanded toward the summit, pubescent like the stem; sepals linear-subulate, spreading at anthesis, 10 mm. long, thinly pubescent; petals bright magenta, 2 cm. long; fertile stamens 5, equal, the anthers lance-subulate, prolonged into a flattened, slightly curved beak with a single apical pore, the connective prolonged at base and minutely bituberculate on the anterior side; sterile stamens reduced to linear-filiform sterile filaments; ovary free, glabrous; style nearly straight, the stigma minutely capitate.

Type, *Hitchcock 16,985*, collected on the East Coast Water Conservancy, southeast of Georgetown, British Guiana, about sea-level, 27 Nov. 1919, and deposited in the herbarium of the New York Botanical Garden. The necessary dissections from which the description was drawn were made from a bud, in which the anthers were 5 mm. long, including the beak of 1.1 mm. Obviously a member of the section *Isostemones*, our species appears most closely related to the last three species of Cogniaux's monograph, and differs from *R. Glazioviana* Cogn. and *R. serrulata* (Rich.) DC. in its broader leaves, and from *R. parviflora* Cogn. in its smaller leaves and much larger flowers.

Siphanthera alsinoides n. sp. Annual, 10–15 cm. high; stems herbaceous, sharply 4-angled or narrowly 4-winged, slender, sparsely hispidulous and glandular-villous, the internodes 18–40 mm. long; petioles flattened, 1–3 mm. long, pubescent like the stem; leaf-blades thin, broadly ovate, 5–9 mm. long, 5–7 mm. wide, obtuse or subacute, crenate-serrulate, especially distally, rounded or subcordate at base, sparsely hispid above with stout, subulate, somewhat paleaceous hairs 0.6–1 mm. long, beneath pale green, glabrous on the surface, minutely and sparsely villous on the veins, obscurely 3-nerved; inflorescence a terminal, sympodial, bracted cyme, each axis ending in a flower subtended by 2 bracts, from the axils of which secondary axes arise, 2–4 cm. long; bracts ovate, sessile, acute, 2–3 mm. long; pedicels sharply 4-angled, 0.7 mm. long; flowers 4-merous;

hypanthium oblong-campanulate, 1.8 mm. long, 1.1 mm. in diameter, pale brown, almost scarious, obscurely 8-nerved, sparsely glandular-villous with hairs 0.4 mm. long; sepals scarious, triangular, 1.5 mm. long by half as wide at the base, sharply acute, reticulate-veined, glabrate; petals rose-pink, apparently fugacious, 3 mm. long, obovate, narrowed to the base, glabrous; stamens 8, dimorphic; fertile stamens: anthers broadly oblong, 0.7 mm. long, 0.5 mm. wide, deep violet, rounded at both ends, glabrous, the rostrum pale violet, terete, 0.2 mm. long and in diameter, obliquely truncate with an introrse pore; connective slender, violet, the basal prolongation 0.3 mm. long, yellow, expanded at the apex into 2 anterior, semicircular appendages, 0.2 mm. long and a little wider; filaments violet-brown, very slender, 1.8–2 mm. long; sterile stamens: anthers yellow, linear-fusiform, 0.3 mm. long; connective not prolonged; filaments 1.6 mm. long; ovary free, ellipsoid, 1 mm. long, glabrous, 2-locular; style straight, 3.7 mm. long, 0.1 mm. in diameter, minutely fusiform-dilated near the apex; stigma spherical, 0.2 mm. in diameter; fruiting hypanthium 3 mm. long, prominently nerved; capsule oblong-cylindric, 2.5 mm. long, depressed at the apex, glabrous, loculicidal; seeds ellipsoid, 0.5 mm. long. FIG. 4, A.

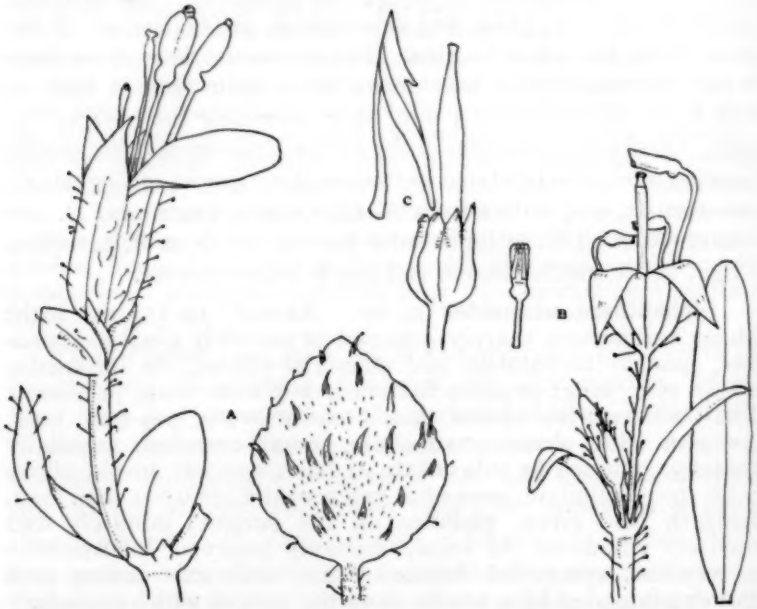

FIG. 4. A. Siphanthera alsinoides Gleason, with foliage leaf, × 7.5. B. Poteranthera minor Gleason, flower, leaf, and anterior face of anther, × 7.5. C. Centronia mutabilis Gleason, pistil and detached stamen, × 2.

Type, *Pennell 1467*, collected in a moist depression in the llano east of Villavicencio, Intendencia Meta, Colombia, alt. 450 m., 26–31 Aug. 1917, and deposited on the herbarium of the New York Botanical Garden. The color of the anthers is taken from the dried specimen: the collector noted them as pink-purple, white at the base.

Since Cogniaux' treatment of *Siphanthera* in his monograph over twenty years ago, no additional species has been described, nor have any been reported from as far west as Colombia. Our plant presents precisely the staminal structure of the genus as hitherto known, but differs from the other species in its cymose inflorescence.

Poteranthera minor n. sp. Herbaceous annual, 5–10 cm. tall, simple or sparingly branched; stems sharply 4-angled, glabrate below, sparsely hispidulous and glandular-villous above, increasing toward the apex, the upper internodes 1–2 cm. long; leaves sessile, crowded at the base of the stem, linear, 6–10 mm. long, 1–1.5 mm. wide, the upper smaller and bractlike, acute, rarely spinulose-ciliate, glabrous on both sides, obscurely 1-nerved; flowers solitary in the upper axils and appearing cymose from the shortening of the terminal internodes, 4-merous; bracts resembling the leaves, 3–5 mm. long, somewhat glandular-villous; pedicels 2–3.5 mm. long, densely glandular-villous; hypanthium campanulate, 1.6 mm. long, 1.1 mm. in diameter, sparsely glandular-villous with violet hairs 0.6–0.8 mm. long, 8-nerved; sepals membranous, triangular, 2.5 mm. long, 1 mm. wide at base, acuminate to a subulate tip, very sparsely glandular-villous, obscurely nerved; petals deciduous, pink-purple; fertile stamens 4, alternate with the petals: anthers oblong, thick, 0.6 mm. long by 0.3 mm. wide and thick, the anther-sacs separate below, connivent above, very slender, violet-colored, opening each by a terminal pore as wide as the sac; connective thick, gradually widened to the base of the anther, prolonged into a subglobose base 0.4 mm. in diameter and minutely 2-tuberculate anteriorly; filaments straight, filiform, 1.7 mm. long; sterile stamens 4, opposite the petals, the anthers narrowly ovoid, 0.5 mm. long, acuminate, minutely 1-pored, the connective not prolonged, the filaments incurved, filiform, 1.3 mm. long; ovary glabrous, 2-locular, subglobose; style straight, 3 mm. long, filiform, somewhat fusiform-thickened below the summit; stigma capitate, 0.2 mm. in diameter; fruiting hypanthium somewhat expanded, the sepals persistent; capsule 2.5 mm. long, loculicidal. FIG. 4, B.

Type, *Pennell 1423*, collected in a moist depression in prairie,

Villavicencio, Intendencia Meta, Colombia, alt. 450 m., 26–31 Aug. 1917, and deposited in the herbarium of the New York Botanical Garden. It is a member of the section *Tulasnea*, characterized by 4-merous flowers and clavate style, and differs from the two species hitherto known in various structural features and dimensions, but particularly in its strongly thickened anthers.

Centronia mutabilis n. sp. Arborescent; branches, petioles, lower leaf-surface, peduncles, pedicels, and calyx prominently fulvous-furfuraceous; stems obscurely 4-angled, the internodes 1–2 cm. long; petioles stout, straight, 15–20 mm. long; leaf-blades firm or coriaceous, ovate or ovate-oblong, 8–9 cm. long, 4–5 cm. wide, obtuse or abruptly acute, sharply obcrenulate (about 5 teeth per cm. of margin, 0.5–1 mm. high), obtuse to truncate or barely cordulate at base, minutely furfuraceous above when young, dark green and glabrous at maturity, 7-pli-nerved, the outer pair obscure and marginal, the transverse veins prominent beneath, about 2 mm. apart, the veinlets minutely reticulate under the lens; leaves of the floriferous branches smaller, 4–6 cm. long, 5-pli-nerved; panicles ascending or erect, 8–10 cm. long, about 10-flowered; pedicels stout, 5–9 mm. long; flower-buds 2 cm. long, obovoid, abruptly beaked, the calyx-limb irregularly deciduous about 8 mm. from the base; flowers 6-merous; petals red, changing to blue, obovate-oblong, 25 mm. long, 20 mm. wide, obtuse, minutely carinate at the apex; filaments strongly flattened tangentially, erect, 10 mm. long; anthers subulate, 9 mm. long; the connective prolonged at base into an acute, conical spur 5.5 mm. long, with a much smaller dorsal appendage extending forward; ovary 6 mm. long, the distal half dissected into flat, lance-oblong, setulose lobes with free acuminate tips 1.5 mm. long; style subulate, 12 mm. long. FIG. 4, C.

Type, *Pennell 9385*, collected at "Alaska," above Salento, Dept. Caldas, Colombia, 7 Aug. 1922, alt. 3100-3400 m., and deposited in the herbarium of the New York Botanical Garden. Another collection is *Hazen 9689*, collected at "Cucarronera," New Quindio Trail, Dept. Caldas, 8 Aug. 1922. In superficial appearance and in the structure of the anther-appendage, the species closely resembles *Centronia haemantha* (Planch. & Lind.) Triana, as illustrated in Flore des Serres, plate 924, and in Trans. Linn. Soc. **28:** *pl. 5, fig. 59b*. But the structure of the ovary is distinctly that of a different section of the genus, *Centroniotypus*. Here it finds its nearest relative in *Centronia Mutisii* (Bonpl.) Triana, also from the Quindio region, which differs in its hispid pubescence, larger leaves, longer pedicels, and white petals.

INDEX TO AMERICAN BOTANICAL LITERATURE

1911–1925

The aim of this Index is to include all current botanical literature written by Americans, published in America, or based upon American material; the word America being used in the broadest sense.

Reviews, and papers that relate exclusively to forestry, agriculture, horticulture, manufactured products of vegetable origin, or laboratory methods are not included, and no attempt is made to index the literature of bacteriology. An occasional exception is made in favor of some paper appearing in an American periodical which is devoted wholly to botany. Reprints are not mentioned unless they differ from the original in some important particular. If users of the Index will call the attention of the editor to errors or omissions, their kindness will be appreciated.

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